UNIVERSITY OF LONDON

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Studies on Skeletal Muscle and Autonomic Function in Chronic Heart Failure

PhD Thesis

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...η δουλεια μου δεν ειναι οι αφηρημενες ιδεες, αλλα ν' ακουω τι μου λενε τα πραγματα του κοσμου, να κοιταζω πως συμπλεκουνται με την ψυχη μου και με το σωμα μου, και να τα εκφραζω.

Γιωργος Σεφερης

...my job isn't abstract ideas but to listen to the matters of the world, to see how they entwine with my soul and my body, and to express them.

George Seferis

To my parents

Nikos and Maria

To my sister

Christine

To my godparents

Stavros and Ioanna

Aknowledgements

I gratefully acknowledge the continuous advices, support, encouragement, monumental patience (!) and above all friendship of Andrew Coats. He has been a *conditio sine qua non* for the undertaking of the odyssey and the permanent light-house to get through this unique adventure.

I would like to especially thank Professor Peter Sleight and Dr James Conway for excellent advices and substantial helps during my time in Oxford, Professor Philip Poole-Wilson for fruitful discussions during my time in London and Professor Dimitrios Kremastinos for encouraging and facilitating my efforts during my time in Athens. My stay in United Kingdom has been enriched and enlivened by the colleagues and friends I met. Sue Clark, Barbara Casadei, Fiona Kemp, Leonard Arnolda, François Brunotte, Alastair McCance, Patrick Davey, Colin Forfar, Graham Kemp, Theo Meyer, Massimo Piepoli, Alberto Radaelli, Thanos Pipilis, Evangelos Pissimissis, Fangxia Qiang, George Radda, Bheeshma Rajagopalan, John Stratton and Campbell Thompson in Oxford together with Elena Cerquetani, Federica del Monte, Piotr Ponikowski, Giuseppe Rosano and Maurizio Volterrani in London have all been wonderful to work with and I am indebted to them all for their invaluable help. Rosemary Bird and Mavis Van der Putt also deserve particular thanks for their technical assistance and unparalleled tolerance throughout the entire study period.

Certain sections of this thesis would have not been possible without the particular expertise and unique atmosphere of collaboration of Jim Johnston and Luciano Bernardi.

Final appearance of text, tables and figures reflect the artistic expertise and computing experience of George Karavolias.

My final and perhaps most important thanks go to my patients. Their contribution was of paramount importance and I would like to express them my gratitute for their impressive patience.

Declaration of Contribution

Two memorable cities hosted my research activities constituting this Thesis: Oxford (John Radcliffe Hospital and Radcliffe Infirmary) and London (Royal Brompton, National Heart & Lung Institute).

All research ideas were originated and materialised by the excellent and inspired collaboration between Professor Andrew Coats and myself. My role was essential in the design of all studies included in the Thesis. I also performed meticulous analysis of all data and contributed substantially to the interpretation and final conclusion of the analysis-arised findings.

I would like, however, to emphasise that an important part of my research work was performed with the help of other investigators.

As far as my contribution to the various techniques used to assess autonomic function and skeletal muscle metabolism is concerned:

Peak oxygen uptake (VO_2max) during the various phases of the studies (baseline assessment, after training and after detraining, as well as before and after dobutamine infusion) was obtained by me by using cardiorespiratory exercise test.

Electrocardiographic recordings and data analysis concerning heart rate (both daytime and nocturnal) and heart rate variability (both in time and frequency domain) were performed to a great extent by me, sometimes with the technical assistance of Jim Johnston or Drs Massimo Piepoli and Piotr Ponikowski.

Noradrenaline kinetics were assessed at Dr Forfar's laboratory in Oxford (John Radcliffe Hospital). Tritiated noradrenaline was infused and blood samples both at rest and serially during either exercise or dobutamine infusion were collected and centrifuged by me, sometimes with the assistance of sister Katherine Prior or Dr Evangelos Pisimisis.

Magnetic resonance spectroscopy human studies took place in Oxford either "up-hill" (John Radcliffe Hospital) or "down-hill" (Radcliffe Infirmary) to evaluate calf muscle or forearm metabolism respectively. Patients were recruited, consented to participate in magnetic resonance spectroscopy studies and supervised during the magnetic resonance spectroscopy studies-related exercise tests by me. Collection of spectra was performed by the experts Dr Bheeshma Rajagopalan, Dr Graham Kemp and Jeff Dunn with the technical assistance of Dr Leonard Arnolda.

Magnetic resonance spectroscopy animal studies took place in Oxford (South Park, Department of Biochemistry). Professor François Brunotte, assisted by Dr David Lindsay and myself, anaesthetised the rats, stimulated their sciatic nerve and collected the spectra.

An important, therefore, part of my research work was realised with the invaluable help of other investigators, to whom I am grateful.

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Overview of the Thesis

Human and animal studies on skeletal muscle and autonomic function in chronic heart failure, with particular emphasis on the role of exercise training, are discussed.

Initially the complex syndrome of chronic heart failure is described including: definitions, epidemiology, aetiologies and pathophysiological characteristics focusing on both cardiac and non-cardiac changes. In particular, neuroendocrine excitation, characterised by activation of the sympathetic nervous system associated with a parasympathetic withdrawal, activation of the renin-angiotensin-aldosterone system, the arginine-vasopressin system, various endothelins as well as the counteracting atrial and brain natriuretic peptides, and musculoskeletal abnormalities involving structure, function and metabolism are reviewed. In additon, the effects of pharmacological and especially non-pharmacological (exercise training) interventions on autonomic balance and muscle metabolism in chronic heart failure are reported.

Methodological aspects are subsequently discussed regarding assessment of sympatho-vagal balance and bioenergetic interpretation of the skeletal muscle metabolic changes during exercise in experimental and human chronic heart failure. Skeletal muscle metabolism is evaluated by using ^{31}phosphorus magnetic resonance spectroscopy, which provides the opportunity of a serial non-invasive assessment of inorganic phosphate, phosphocreatine, ATP levels and intracellular pH, all indices of glycolytic activity and mitochondrial oxidative capacity, both at rest and during exercise as well as during the recovery period. Sympatho-vagal balance is assessed by using heart rate variability measures and radiolabeled noradrenaline kinetics. Measures of heart rate variability in the time (standard deviation of R-R intervals) and frequency (power spectral analysis-derived low- and high-frequency components of heart rate variability) proved to be useful clinical tools for semi-quantitative assessment of sympatho-vagal balance and are widely used in our studies. Radiotracer kinetic techniques, using infusions of [^{3}H] noradrenaline, enable us to estimate whole-body noradrenaline spillover to plasma (the overall rate at which noradrenaline released from nerve endings enters plasma) and whole-body noradrenaline plasma clearance simultaneously. Thus, we avoid the confounding influence of noradrenaline plasma clearance, which is reduced in severe chronic heart failure, when we simply measure plasma noradrenaline concentration as an index of sympathetic nervous activity. Phosphorus-31 magnetic resonance spectroscopy studies of skeletal muscle metabolism in heart failure have shown increased phosphocreatine breakdown and intracellular acidosis during exercise, both in human subjects as well as in rats following a large myocardial infarction. This increase in phosphocreatine breakdown and intracellular acidosis implies an increased glycolytic contribution to the required ATP synthesis, due either to an increase in the requirements for ATP (resulting perhaps from muscle atrophy or

decrease in metabolic efficiency), to a defect in oxidative ATP synthesis, or to a primary alteration in the balance between glycogenolytic and oxidative ATP synthesis. Skeletal muscle metabolic changes were examined in the gastrocnemius muscle at rest and during exercise in patients with chronic heart failure and in healthy control subjects to look at the effects of physical training on skeletal muscle metabolism in heart failure, in the dominant forearm muscle at rest and during exercise in patients with extensive anterior myocardial infarction to describe the time course of skeletal muscle metabolism following first large anterior myocardial infarction and in the calf muscles during sciatic nerve stimulation at 2 Hz in a rat model with myocardial infarction to study the influence of exercise training and infarct size on muscle metabolism in experimental heart failure. Phosphocreatine recovery following exercise was also analysed, which has been proposed as a measure of muscle oxidative capacity that is independent of muscle mass, recruitment and workload. More specifically the end-exercise adenosine diphosphate concentration and initial phosphocreatine resynthesis rate were used to calculate the *maximum rate of oxidative ATP synthesis*, which is a quantitative measure of mitochondrial capacity (a function of mitochondrial content, mitochondrial activation state and blood flow). Another inverse measure of mitochondrial function, the half-time of phosphocreatine recovery, was calculated from the slope of a semilogarithmic plot. The sum of glycogenolytic ATP synthesis rate and the initial rate of phosphocreatine depletion was also used to estimate the *initial rate of ATP turnover*, which is equivalent, in practice, to the initial ATPase rate measured by the very early rate of phosphocreatine depletion. For a given initial power output, the *initial rate of ATP turnover* is inversely proportional to muscle mass and to metabolic efficiency, and for present purposes these parameters were taken together as the *effective muscle mass*.

To quantify the reproducibility of heart rate variability measures, standard deviation of R-R intervals together with low- and high-frequency components of heart rate variability (by autoregressive spectral analysis) were calculated from short-term sampling periods. To this end 10 patients with chronic heart failure were evaluated during stable conditions and during two different sympathetic stimulations: inotrope (dobutamine) infusion and physical exercise. Our data indicate that the reproducibility of heart rate variability parameters is reasonable, although not particularly high at the higher levels of sympathetic stimulation.

In an attempt to evaluate the ability of different methods to describe autonomic function in chronic heart failure 25 patients with moderate to severe chronic heart failure were studied before and after 8 weeks of physical training at home. Sympatho-vagal balance was assessed by 24-hour daytime and nocturnal heart rate, submaximal heart rate during bicycle exercise, heart rate variability in the time (standard deviation of R-R intervals) and frequency (low- and high-frequency components of heart rate variability) domain and radiolabeled noradrenaline spillover. Results show a lack of correlation between methods describing autonomic balance in chronic heart failure, indicating that a comprehensive description of the autonomic status may necessitate a panel of complementary methods.

Human and animal studies examine the role of physical training programmes on skeletal muscle metabolism in experimental and human heart failure, evaluate the effects of physical training on autonomic balance (paying specific attention on the circadian pattern of heart rate variability before and after training) in stable chronic heart failure, assess the effects of inotrope 'training' (by pulsing ß-stimulant therapy) on exercise performance, ß-adrenoceptors density and chronotropic responsiveness in patients with chronic heart failure and finally describe the time course of central haemodynamics, autonomic function and skeletal muscle metabolism in patients following extensive anterior myocardial infarction:

I. Firstly, *studies on skeletal muscle and autonomic function in chronic heart failure* describe the skeletal muscle metabolic abnormalities characterising the complex syndrome of chronic heart failure and examine the effects of exercise training programmes on skeletal muscle metabolism in experimental and human heart failure.

Recent investigations have established the presence of intrinsic skeletal muscle metabolic abnormalities in chronic heart failure, thus explaining, at least partially, the lack of correlation between exercise performance and degree of left ventricular dysfunction. Muscle deconditioning is a possible mechanism underlying impaired skeletal muscle myopathy characterising patients with chronic heart failure. The influence of physical training on skeletal muscle metabolism was studied both after myocardial infarction in a rat model of the development of heart failure and in patients with moderate to severe chronic heart failure. Phosphorus-31 magnetic resonance spectroscopy and enzyme assays were performed in female wistar rats 12 weeks after coronary artery ligation and in a non-trained sham-operated control group. Infarcted rats were radomly allocated to either 6 weeks of training or non-training. Phosphorus-31 magnetic resonance spectroscopy was also used to study muscle metabolism during exercise in 12 patients with ischaemic chronic heart failure who underwent 8 weeks of home-based bicycle exercise training in a randomised crossover controlled trial. Phosphorus-31 spectra were collected from the calf muscles of both rats and patients (at rest, during sciatic nerve stimulation and during incremental-workload plantarflexion respectively, and during recovery from either stimulation or exercise) to evaluate changes in muscle pH and in the concentrations of phosphocreatine and adenosine diphosphate. In addition, fibre typing and enzymatic assays were performed on the calf muscles of the contralateral non-stimulated leg in rats to measure the mitochondrial oxidative enzymes citrate synthase and ß-hydroxyacyl CoA dehydrogenase and the mitochondrial-cytoplasmic enzyme glutamate pyruvate transferase.

Evidence is presented that rats with congestive heart failure developed similar skeletal muscle metabolic changes in the handling of high energy phosphates to those described in humans with heart failure and that physical training programmes in rats and in patients with congestive heart failure can achieve a substantial correction of the impaired oxidative capacity of skeletal muscle.

II. Secondly, *studies on skeletal muscle and autonomic function in chronic heart failure* throw light on the role of exercise training on the autonomic function in human chronic

heart failure focusing, also, on the effects of training on the circadian pattern of heart rate variability parameters describing sympatho-vagal balance.

Physical deconditioning may cause or perpetuate some of the secondary changes observed in chronic heart failure. These include excessive neurohormonal vasoconstrictor activity and alterations in autonomic control mechanisms (sympathetic predominance associated with parasympathetic withdrawal), which may exacerbate symptoms and effort intolerance. The effects of an exercise training programme on the autonomic function were studied in 25 patients with moderate to severe chronic heart failure (NYHA II-III), randomised to 8 weeks of home-based bicycle exercise (20 minutes per day, 5 days per week at 70-80% of their maximal heart rate) or avoidance of exercise in a crossover design.

A well-defined diurnal pattern of dynamic changes in sympatho-vagal balance has been recently linked with the circadian variation of acute cardiovascular events. The effect of exercise training on the circadian pattern of heart rate variability, recorded over 24 hours in relation to both time and frequency, was studied in 12 patients with stable moderate to severe chronic heart failure, randomised in a crossover design to 8 weeks training or detraining, and compared with an age-sex matched control group of 12 normal subjects. The circadian pattern of heart rate variability was assessed by calculating low- and high-frequency power and their ratio for each hour.

The possibility is discussed that the autonomic imbalance associated with chronic heart failure may in part be due to chronic physical deconditioning and may, at least partially, be reversible by exercise training programmes in carefully selected patients. Moreover, the circadian variations in autonomic parameters appeared to be preserved in chronic heart failure in both training and detraining conditions.

III. Thirdly, *studies on skeletal muscle and autonomic function in chronic heart failure* attempt as closely as possible to imitate pharmacologically the stimulus to the β_1-receptors of the cardiac myocyte produced by physical training.

Physical training can improve symptoms, exercise performance, autonomic function and skeletal muscle metabolism in patients with chronic heart failure and the possibility has been discussed that pharmacological therapy may be able to simulate some of these benefits. Short (20-30 minutes) periods of high-level exercise were replaced with short bursts of pharmacological β-adrenergic stimulation with dobutamine (sufficient to raise heart rate to 70-80% maximal and maintain the infusion for 30 minutes on 4 days per week) in an attempt to determine whether short duration pulsed inotropic therapy induces a pharmacological conditioning effect in 10 patients with stable moderate to severe chronic heart failure. Results were compared with a control group of 10 patients matched for age and severity where no dobutamine infusion was performed. Lymphocyte β-receptor density, autonomic control (assessed by heart rate variability in the time and frequency domain and by noradrenaline plasma levels) and exercise tolerance were the major end points, which reevaluated immediately and 6 weeks after the completion of pulsed inotrope therapy and in the control group of patients.

Results encourage us to reconsider the role of ß-receptor stimulants because short duration pulsed inotrope therapy induces pharmacological conditioning with improved symptoms and exercise tolerance associated with improved autonomic balance, ß-receptor up-regulation and enhanced chronotropic responsiveness in patients with chronic heart failure. Evidence is reported that these beneficial effects persist for at least 6 weeks after pulsed inotrope therapy.

IV. Finally, *studies on skeletal muscle and autonomic function in chronic heart failure* enable us to obtain a better understanding of the genesis and prognostic significance of skeletal muscle and neurohormonal abnormalities in asymptomatic left ventricular dysfunction by studying their evolution after myocardial infarction.

The time course of skeletal muscle metabolic changes and autonomic function following extensive anterior myocardial infarction was described in 10 patients, using ^{31}phosphorus magnetic resonance spectroscopy to study forearm metabolism, heart rate variability (in the time and frequency domain) and radiolabeled noradrenaline kinetics to assess sympatho-vagal balance and pulsed-wave Doppler to estimate cardiac output. Results were compared with 22 normal subjects and 22 patients with stable chronic heart failure. Studies were performed at 1-3 weeks ('early'), 6-8 weeks ('mid') and 6-9 months ('late') following a first extensive anterior myocardial infarction.

Data emerging from the study suggest that skeletal muscle metabolism and autonomic function become abnormal after an extensive myocardial infarction, although they do not follow similar patterns. Skeletal muscle metabolic abnormalities are slow to develop and unrelated to the degree of failure, whereas early excessive neurohormonal activation seem to characterise patients who subsequently develop chronic heart failure.

Chapter I

Introduction

A. Epidemiological Features of Chronic Heart Failure

I. Definition-Aetiology

No universally agreed definition of heart failure has been given; 'heart failure' itself is considered a rather unfortunate term encompassing negative connotations for the patient and describing imprecisely several different clinical conditions. Subdivisions on the basis of presumed pathophysiological mechanisms such as: a) 'forward' or 'backward', b)'congestive' or 'non-congestive' and c) 'high-output' or 'low-output' have not proved to be particularly useful in both clinical and therapeutic terms. Also, left and right heart failure, although quite distinct clinical entities, they are rather misleading since they frequently coexist in the clinical syndrome of biventricular failure and the commonest cause of right heart failure is damage to the left ventricle (Poole-Wilson, 1996). A relatively more useful classification has been recently introduced depending on the predominant pattern of left ventricular dysfunction: systolic, diastolic or mixed.

Difficulties in describing this complex clinical entity can be, partially, attributed to the prevailing view during the last 20 years according which physicians had generally regarded heart failure as a haemodynamic disorder in an attempt to explain patients' symptoms and disability. It was only after the development of the neurohormonal hypothesis 18 years ago (Packer, 1992a) and more recently the evolution of muscle hypothesis (Coats *et al.*, 1994; Piepoli *et al.*, 1996) to explain the progression of chronic heart failure (CHF), that heart failure is considered a syndrome and not a single diagnosis. Heart failure, therefore, is a clinical syndrome which develops as a consequence of cardiac disease and is recognised clinically by a constellation of symptoms and signs (readily diagnosed by doctors) produced by complex circulatory (haemodynamic, renal, vascular), neurohormonal and skeletal muscle responses to cardiac dysfunction (Poole-Wilson, 1996). Whatever the complexities of the ventricular pathophysiology that initiates events, the syndrome a) may occur as the end-result of damage caused by a number of disease processes (coronary artery disease, viral infection, alcohol misuse, hypertension or valvular defects) leading to reduced ventricular (usually left) contractile reserve, b) is characterised by symptoms (dyspnoea and/or muscle fatigue) either at rest or at unexpectedly low level of exercise and c) is associated with biochemical, hormonal, metabolic and functional alterations in many disparate organ systems. Extreme volume overload (endotoxic high-output shock, severe anaemia, arteriovenous fistulae or shunts) and pressure overload (acute hypertensive crisis, prosthetic valve occlusion) conditions can produce a clinical picture similar to that of heart

failure when ventricular function itself is normal and should, therefore, be separated (for clinical and research purposes) from cases where the initiating cause is ventricular dysfunction. The presence and severity of cardiac failure can be assessed by several methods, such as symptom questionnaires, physical and radiographic examination and by measures of ventricular and exercise performance, all of which have significant limitations when used independently, especially when the syndrome is mild (Hlatky *et al.*, 1986; Chakko *et al.*, 1992).

The clinical syndrome of heart failure is the common end-result of many different disease processes that impair myocardial function. Coronary artery disease and hypertension (either singly or together) as well as idiopathic cardiomyopathy account for the vast majority of cases of heart failure within the developed world. Coronary artery disease seems to account for about half of the cases of cardiac failure depending on age and sex (Kannel *et al.*, 1994). The Framingham study suggested that hypertension, especially when complicated by left ventricular hypertrophy, was by far the most common underlying cause of heart failure; this study reported hypertension as the sole or contributory cause of heart failure in over 70% of cases (Kannel *et al.*, 1972). Most recent intervention trials, however, have included a preponderance of patients with heart failure secondary to ischaemic heart disease and, in cross-sectional studies in the community (Eriksson *et al.*, 1988; Remes *et al.*, 1992; Parameshwar *et al.*,1992) hypertension is considered a relatively minor cause of heart failure. This may be partly due to better detection and treatment of hypertension but may also reflect either relabelling (misclassification, in the Framingham's non-invasive era, in terms of aetiology because of the frequent coexistence of hypertension with ischaemic heart disease) or 'burnt out' hypertension (normotensive patients with the only clue to the true hypertensive antecedent of heart failure being the greater than expected left ventricular hypertrophy). In industrialised societies, previously common causes such as nutritional deficiency or rheumatic valvular heart disease are now rather rare; they are still common in the developing world and consequently the prevalence and incidence of CHF in younger age groups is higher in less developed societies (Johnson *et al.*,1982; Killip, 1985; Kannel *et al.*, 1994). Particular underlying causes, including Chagas' disease in Central and Southern America, iron overload in certain tribes in Southern Africa and nutritional deficiency in the world's poorest countries, should always be suspected in assessing patients from these regions. Among 220 patients who met the case definition of new heart failure over a 20 month period, on the basis of clinical assessment, electrocardiography, chest radiography and transthoracic echocardiography, the primary aetiologies were: coronary heart disease (36%), unknown (presented with a dilated globally hypokinetic left ventricle, 34%), hypertension (14%), valve disease (7%), atrial fibrillation alone (5%), and other (including alcohol, 5%) (Cowie *et al.*,1999). Atrial fibrillation is the cardiac rhythm in 31% of patients presenting with heart failure for the first time (Cowie *et al.*, 1999). Although the possibility of underlying myocardial disease (of unknown aetiology) presenting with atrial fibrillation cannot be excluded, in a minority of patients, especially in an elderly heart lacking myocytes and with increased interstitial fibrosis due to age-related changes (Morley *et al.*, 1989), the

onset of atrial fibrillation may be sufficient to cause heart failure in the absence of concomitant coronary artery disease or hypertension and left ventricle not more than mildly hypokinetic whilst in fast atrial fibrillation.

II. The Incidence of Clinical Heart Failure

Despite important reductions in cardiovascular mortality that have occured over the past three decades in most industrialised regions, CHF is the only common cardiovascular condition that is increasing in incidence, prevalence and mortality and remains responsible for major human and economic costs (Massie *et al.*,1996). As premature deaths from these conditions are avoided by means of more effective medical and surgical therapeutic interventions, the prevalence of CHF is expected to rise because the predisposing conditions

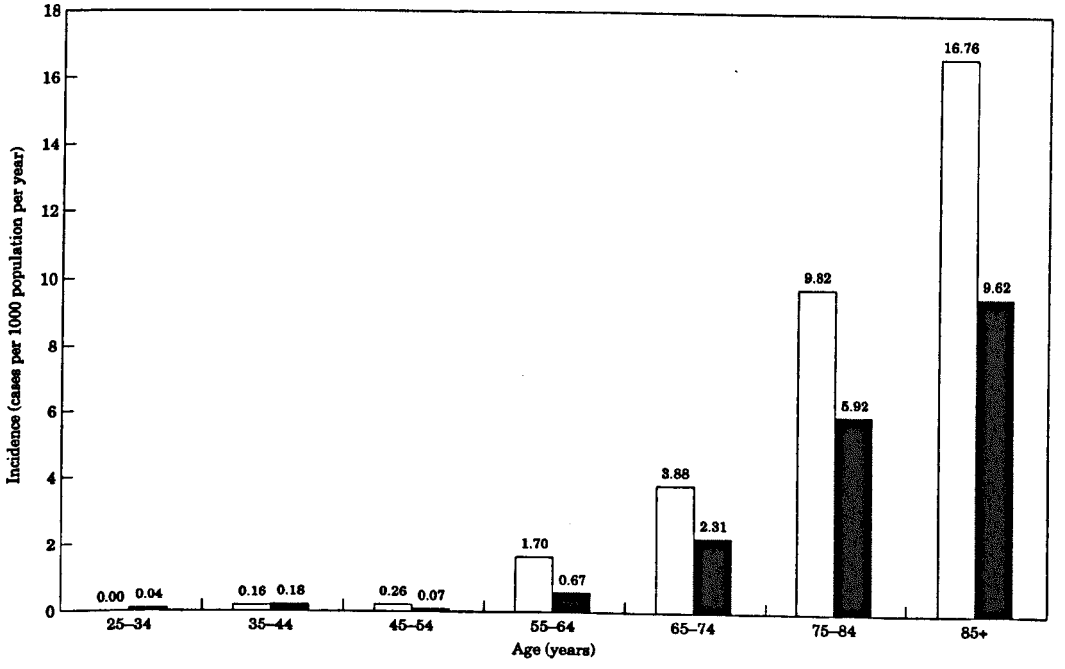

Figure 1.1. Incidence of clinical heart failure by sex and age group, (adapted from Cowie *et al.*, 1999). Note the higher incidence in males than females and in those aged 75 years or over.

are palliated, not cured and because the elderly population is also increased in size. Improvements, for example, in the management of acute myocardial infarction and chronic coronary artery disease has led to more heart failure, as more people survive to develop this syndrome later in life. Thus, general practitioners in London reported a prevalence of CHF in 4 out of every 1000 patients in the general population (Parameshwar *et al.*, 1992). Moreover, the most striking and consistent observation is the highly significant relationship between age and the occurence of CHF: prevalence rises with age from 1% of the population aged 25 to 54 years to 4.5% at 65-74 years (Schocken *et al.*, 1992). Prevalence estimates vary widely reflecting the differences in methodology and timing rather than true differences between populations and the crude prevalence for those aged over 65 years ranges from 3 to

13% (Cowie *et al.*, 1997). It is probably nearer to 10% in those >75 years (Kannel *et al.*, 1994).

Incidence of CHF can be better determined by a population-based surveillance system in which subjects developing this syndrome for the first time are identified prospectively when they present to the health care system (either in primary or higher levels of care). Thus, national statistics (Lamberts *et al.*, 1993; Van de Lisdonk *et al.*, 1990; Remes *et al.*, 1992; Rodeheffer *et al.*, 1993; Royal College of General Practitioners, 1995) based on admissions to hospital and visits to clinics indicate a high incidence of CHF which ranges in the general population from 1.0 to 5.0 cases per 1000 population per annum, with a steep increase with advancing age (Figure 1.1): 0.02 per 1000 population per annum in those aged 25 to 34 years to 11.6 in those aged 85 years or over and higher in males than females (age-standardized incidence ratio 1.75) (Cowie *et al.*, 1999). In the population above 75 years of age the incidence rate is reported to be as high as 40 cases per 1000 population per annum in some studies (Lamberts *et al.*, 1993; Van de Lisdonk *et al.*, 1990). In the Framingham Study the incidence of CHF doubles with each decade of age with only slight male predominance (Kannel *et al.*,1994). There were 722 000 hospital discharges in the United States in 1990 with CHF as primary diagnosis, and over two million if secondary diagnoses are also included, representing the most common diagnosis on discharge from hospital in the United States in people over the age of 65, and the second most common overall, namely a fourfold increase of admissions to hospital since 1971 when 165 000 were so listed, thus indicating a rapidly expanding problem as the population ages and increases in size (Graves, 1991). Data from hospital discharge statistics, not including outpatient visits or home health care, estimate the number of visits to physicians' clinics in the United States in 1989 as high as 2 340 000 (Graves *et al.*,1991; National Heart, Lung and Blood Institute, 1992). Chronic heart failure is the second most frequent cardiovascular diagnosis during office visits in the United States (an estimated 11.4 million diagnoses in 1990) (O'Connell *et al.*, 1994). Extrapolation of the average annual incidence of CHF from the Framingham Study to the population of the United States provides an estimate of 465 000 new cases of cardiac failure each year (National Heart, Lung and Blood Institute, 1992). The expenditure on hospitalisations for CHF were more than twice the expenditure for all types of cancer and substantially more than that spent on myocardial infarction.

III. Survival-Prevention

Although medical interventions, such as angiotensin-converting enzyme (ACE) inhibitor therapy (The Consensus Trial Study Group 1987; The SOLVD Investigators 1991) and more recently therapy with either selective antagonists of β_1 adrenoceptors (MERIT-HF and CIBIS-II studies, 1999) or new generation of β-blockers (Packer *et al.*, 1996), can be beneficial in patients with CHF, as with any epidemic, prevention is the most effective approach to improve outcome. Given that the major risk factors for CHF are atherosclerotic vascular disease and hypertension, aggresive antihyperlipidaemic and antihypertensive therapy prevents the development of heart failure (Veterans Administration Cooperative

Study Group 1970; SHEP Cooperative Research Group 1991). In patients with asymptomatic left ventricular dysfunction pharmacological (notably ACE inhibitors) and non-pharmacological (such as physical conditioning) interventions have been proposed to prevent or delay the onset of CHF and improve prognosis with some success (The SOLVD Investigators 1992; Pfeffer *et al.*, 1992; Specchia *et al.*, 1996). Approximately 50% of all deaths in those who developed CHF are sudden, whereas the majority of the remainder is due to progressive pump failure (Kannel *et al.*, 1988; Bigger, 1987). With the possible exception of amiodarone in severe CHF and still questioning the preventing role of ß-blockers and AT-II antagonists, current antiarrhythmic therapy and ACE inhibitors have little or no effect on the number of sudden deaths offering their prognostic benefit in recent heart failure trials through a reduction in the number of deaths from progressive pump failure (Stevenson *et al.*, 1993; Doval *et al.*, 1994; Garg *et al.*, 1995).

Information on the prognosis of heart failure can be derived from population-based studies, hospital series and the placebo arm of heart failure trials. The Framingham Heart Study reported a mortality rate higher than that described in the trials of therapy in mild to moderate CHF with a median survival of only 1.66 years in men and 3.17 years in women. Excluding mortality in the first 3 months (as is the case in most clinical trials) median survival is still only 3.2 years in men and 5.4 years in women (Ho *et al.*, 1993b; Kannel *et al.*, 1994). Therefore, once heart failure had developed only 25% of men and 38% of women were alive 5 years later, reflecting a mortality rate 6 to 7 times that of general population of the same age, an outlook for CHF proved to be even more lethal than many malignancies (Ho *et al.*, 1993a). The poor prognosis of heart failure was confirmed in the study in Rochester, Minnesota (another population-based study), where only 66% of patients were alive 1 year after the diagnosis of the syndrome (Rodeheffer *et al.*, 1993). Hospital series and the placebo group of pharmacological trials, although reflecting the more severe cases or a highly selected group of patients with CHF respectively, both confirm the marked reduction in life expectancy at any age. Thus, several hospital-based series of patients with CHF refractory to standard medical therapy reported (Wilson *et al.*, 1983b; Franciosa *et al.*, 1983) a high mortality rate ranging from 34 to 48% at 1 year, 59 to 68% at 2 years and 76% at 3 years. Similarly, the first CONSENSUS Study, enrolled only patients with severely symptomatic heart failure, reported a mortality rate of 52% at 1 year in the placebo group (The Consensus Trial Study Group, 1987). Survival is adversely affected by age: the Framingham study reported a 27% increase in mortality rate per decade of advancing age in men and a 61% gradient in women (Ho *et al.*, 1993b). This has not been confirmed by other studies (Wilson *et al.*, 1983b; Gradman *et al.*, 1989). Framingham data, however, may be closer to the reality because of the longer follow up period and complete inclusion of all types of heart failure from the time of onset. At all ages the death rate is higher in black than white patients with CHF, which may be related to the increased prevalence of hypertension and early coronary artery disease in the black population (Burt *et al.*, 1995). Based on the Framingham Study data the considerable decline in mortality from coronary artery disease was not associated with impressive change in prognosis of CHF over 4

decades of observation. In the United States the number of deaths ascribed to CHF as either the underlying or contributing cause of death rose from 130 000 in 1970 to 267 000 in 1988 (Kannel *et al.*, 1994; Cowie *et al.*, 1997). Although part of this increase may be explained by the increasing proportion of the elderly within the population, age-adjusted death rates for heart failure as the underlying cause of death increased from 1979 to 1988. The effect, however, of the relatively recent introduction of ACE inhibitor and ß-blockade therapy on survival has not been thoroughly examined as yet, given that after 1988 age standardised rates started to decline (Gillum 1993). A recent meta-analysis demonstrated a significant reduction in mortality of patients recruited in trials of ACE inhibitors in CHF but the gain in life expectancy is estimated in months rather than in years (Garg *et al.*,1995). In addition, a substantial proportion of patients with CHF outside the context of clinical trials are not receiving such optimal treatment (Anonymous, 1992; Clarke *et al.*, 1994).

B. Alterations of Skeletal Muscle in Chronic Heart Failure

The inability of central haemodynamics to explain the generation of symptoms in CHF has shifted the emphasis of attention away from the heart towards non-haemodynamic peripheral factors. Three remarkable observations have recently directed research emphasis to be focused on peripheral adaptations (or maladaptations) to the CHF state that may contribute to the functional condition and exercise limitation: exercise tolerance and symptomatic status in patients with CHF is not closely related to the extent of left ventricular dysfunction (Franciosa *et al.*, 1981) (Figure 1.2); ambulatory pulmonary arterial pressure monitoring has revealed that limiting dyspnoea can occur at totally different peak pulmonary arterial pressures during different types of exercise, thus arguing against pulmonary arterial haemodynamics being the underlying cause of this symptom (Gibbs *et al.*, 1990); an

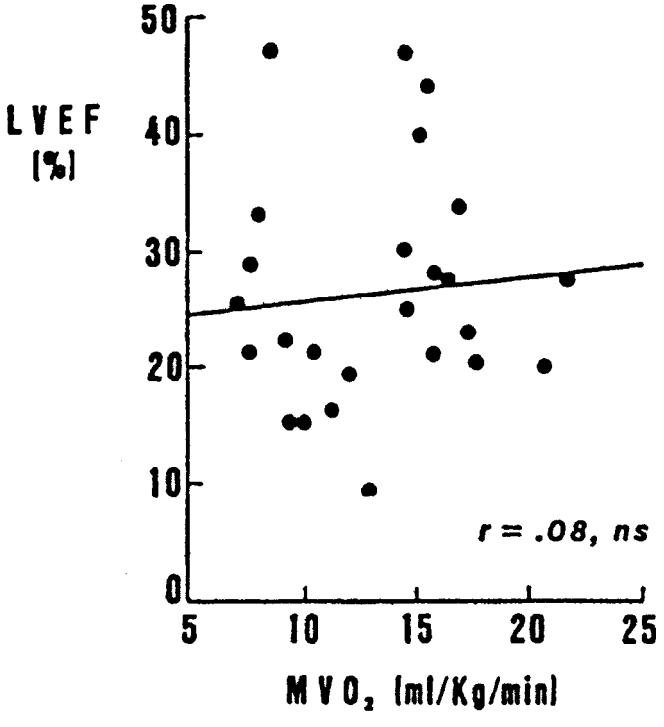

Figure 1.2. Lack of correlation between central haemodynamics and exercise tolerance in chronic heart failure. This plot demonstrates the very poor correlation between left ventricular function, as assessed by ejection fraction, and exercise capacity, measured by peak oxygen uptake (VO_2max) during cycle ergometry (adapted from Minotti *et al.*, 1992a).

acute enhancement of cardiac output achieved by a major haemodynamic intervention, such as cardiac transplantation, does not immediately increase exercise capacity (Sinoway *et al.*, 1988). Peripheral alterations involving skeletal muscle function, metabolism and structure include: reduced peripheral perfusion, reduced muscle mass and intrinsic skeletal muscle metabolic abnormalities. It has proved, however, rather impossible to describe specific pathological changes characteristic of CHF, not only because of the considerable variation in a control population, but also because muscle becomes abnormal in a limited number of ways in a variety of disease entities associated with skeletal myopathy. It should be emphasized that skeletal muscle myopathy can be part of an inherited myopathy affecting both cardiac and skeletal muscle. Skeletal muscle changes, however, are observed in CHF regardless of the underlying aetiology making, therefore, unlikely the hypothesis of an inherited cardioskeletal myopathy causing subclinical skeletal myopathy.

Skeletal muscle abnormalities were first described by the school of Hippocrates around 400 BC who considered CHF as condition in which "... the shoulders, clavicles, chest and thighs melt away". Only in the past ten years, however, skeletal muscle changes have been documented in any detail and their role in the generation of symptoms in patients with CHF has been thoroughly investigated.

I. Peripheral Perfusion

Several studies have shown reduced skeletal muscle blood flow in CHF and documented that reduced blood flow can cause metabolic abnormalities in exercising muscle and that inadequate muscle flow is a key contributor to exertional fatigue. Thus, in a series of studies (Zelis *et al.*, 1968 and 1974; Wilson *et al.*, 1984b), using either venous occlusion plethysmography or venous thermodilution, demonstrated that limb blood flow was reduced during handgrip or bicycle exercise and that, after a period of forearm ischaemia, maximal blood flow was also reduced, associated with increased peripheral vascular resistance. In addition, patients with CHF exhibit impaired arteriolar vasodilation, as evidenced by a failure of limb vascular resistance to decrease normally during exercise (Wilson *et al.*, 1984b; LeJemtel *et al.*, 1986). Three major regulatory systems are activated in CHF, the sympathetic nervous system, the renin-angiotensin-aldosterone system and the arginine vasopressin system, and exert potent constrictor effects on peripheral blood vessels (Packer, 1992a). In addition to these circulating factors, heart failure is accompanied by an enhanced release of locally active vasoconstrictors produced by vascular endothelium (ie. endothelin); endothelin concentrations are increased in proportion to the severity of the syndrome of heart failure (Margulies *et al.*, 1990). Circulating vasoconstrictive neurohormones [endothelin, noradrenaline (NA), renin, angiotensin II, and vasopresssin] and sympathetic vasoconstrictor activity contribute substantially but are not solely responsible for the elevation of peripheral vascular resistance and the reduced response to vasodilator stimuli in CHF. Heart failure syndrome may, also, increase systemic vascular resistance directly by altering the mechanical properties and reducing the dilating ability of the resistance vessels. Thus, fluid and sodium retention can impair arteriolar vasodilation

in humans possibly by tissue compression of capillaries or intrinsic vascular changes (Zelis and Flaim, 1982). Removal, however, of excess fluid and sodium retention does not completely normalise arteriolar vasodilation (Sinoway *et al.*, 1987). It has been, also, suggested that exercise-induced excessive increases in catecholamines and angiotensin II may impair vasodilation. Blocking, however, these systems by ACE inhibitors or *a*-blockers does not restore vasodilation acutely (Zelis *et al.*, 1968; Wilson and Ferraro, 1985b; Wilson *et al.*, 1985a; Drexler *et al.*, 1989), indicating the presence of additional abnormalities including an abnormality of the vascular endothelium or vascular remodeling as a result of a chronic effect of angiotensin II.

Reduction in endothelium derived relaxing factors (EDRFs) (Lüscher *et al.*, 1993) represents an important potential mechanism underlying the reduction in vasodilatory capacity. It is worthmentioning that EDRFs are produced continuously under basal conditions, thus providing a constant force to counteract vasoconstrictive agents such as angiotensin II, NA and endothelin. Endothelium derived relaxing factor is released in response to both neurotransmitters (acetylcholine and bradykinin) and mechanical stimuli (changes in blood flow velocity and endothelial shear stress) (Cooke *et al.*, 1990; Moncada *et al.*, 1991). To a great extent, endothelium-dependent dilation is mediated by EDRF, the biological activity of which is provided by nitric oxide (NO) or by a closely related substance, as evidenced by using N^G-monomethyl L-arginine (L-NMMA) as a selective inhibitor of the production of NO from L-arginine (Palmer *et al.*, 1988; Dinerman *et al.*, 1993).

The reduced NO-mediated vasodilation characterising CHF is multifactorial: i. constitutive nitric oxide synthase (cNOS) is down-regulated as shear stress is reduced (Miller *et al.*, 1988; Cooke *et al.*, 1990), ii. vascular ACE producing angiotensin II (powerful vasoconstrictor) and inactivating bradykinin (potent vasodilator peptide) is up-regulated; angiotensin II inactivates endothelium-derived NO possibly by stimulating NADH/NADPH oxidase systems of smooth muscle cells, whereas bradykinin acts through specific endothelial receptors to cause the release of prostacyclin, NO (by increasing the activity of cNOS) and endothelium-derived hyperpolarising factor (Mombouli *et al.*, 1992; Rajagopalan *et al.*, 1996; Ferrari *et al.*, 1998a), iii. recent investigations have demonstrated that tumor necrosis factor (TNF-a) can be responsible for reduced peripheral vasodilating capacity in CHF due to endothelial dysfunction. Enhanced TNF-a may cause endothelial dysfunction by inducing oxidative stress, which, in turn, neutralises or destroys NO and causes endothelial-cell apoptosis (Ferrari, 1998b). Experimental and clinical data support the concept that ACE inhibitors improve vascular function by increasing bradykinin levels and thereby improving endothelium mediated vasodilation due to enhanced availability of NO (Hornig *et al.*, 1998). Also, physical training programmes improve flow or agonist mediated endothelium-dependent vasodilation, perhaps via an increase in endothelial NO formation and release (Hornig *et al.*, 1996; Hambrecht *et al.*, 1998).

Chronic heart failure is characterised by a near maximal extraction of oxygen by the exercising muscle and ACE inhibition results not in an acute but in a delayed improvement in exercise capacity which is associated with enhancement of peripheral blood flow and

oxygen extraction during exercise, possibly not occuring as a result of an increased capillary density but as a result of restoration of endothelial function (Drexler *et al.*, 1989; Clozel *et al.*, 1991; Herrlin *et al.*, 1991; Schaufelberger *et al.*, 1996).

However, reduced blood flow and therefore muscle underperfusion alone cannot explain the muscle metabolic alterations because plethysmographic measures of forearm blood flow have not been different in patients with CHF and in normal control subjects (Wiener *et al.*, 1986) and because marked differences in skeletal muscle metabolism persist even during ischaemic forearm exercise (Massie *et al.*, 1988). Although there is agreement that in heart failure the total peak blood flow to an exercising limb is reduced, where the flow is expressed per unit muscle volume the results are contradictory, suggesting that an important part of the reduced flow may be due to a deficiency in the total muscle bulk (Coats *et al.*, 1994).

II. Ultrastructural and Cytochemical Alterations

Volume density of mitochondria (mitochondrial volume fraction per unit volume of muscle tissue), cristae surface density (surface fraction of mitochondrial cristae per unit volume of

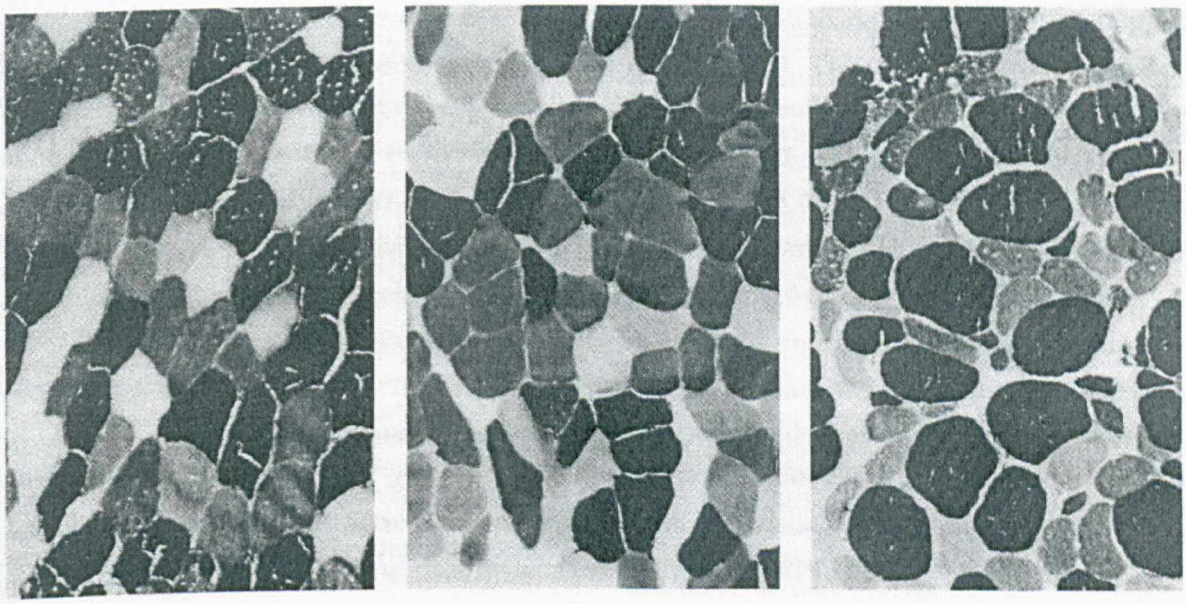

Figure 1.3. Shift in skeletal muscle fibre type distribution from type I oxidative slow-twich to type II fast-twich glycolytic fibres and reduction in the size of both (IIa and IIb) fibre II subtypes in patients with heart failure. Microphotograph of myofibrillar ATPase stain at pH 4.6 for a normal subject (left panel) and two patients with heart failure (middle and right panels). Black-stained fibres are type I, grey fibres type IIb and light fibres type IIa. Type II (grey and light) fibre predominance in patients with chronic heart failure is readily apparent in middle panel. Type II fibre atrophy in patients with heart failure is clearly demonstrated in far right panel, because type II fibres are normally equal to or larger than type I fibres (adapted from Mancini *et al.*, 1989).

muscle) and cytochrome *c* oxidase activity of skeletal muscle mitochondria are substantially reduced in patients with severe CHF irrespective of age and aetiology, indicating an impaired oxidative capacity of working muscle (Drexler *et al.*, 1992). The same investigators demonstrated that the capillary length density of skeletal muscle is reduced in CHF and the

fibre type distribution is shifted from type I oxidative slow-twich to type II fast-twich glycolytic fibres. When type II fibres have been subtyped, the increase in the percentage of type II fibres appears to be due to an increase in type IIb fibres with no alteration in type IIa frequency (Sullivan *et al.*, 1990). Type I fibres have been reported to be reduced. Some investigators (Minnotti *et al.*, 1993b) found that, although the percentage of type I oxidative fibres was reduced, the relative size was larger, whereas others (Lipkin *et al.*, 1988) demonstrated atrophy of both type I and type II fibres. Regarding size of type II fibres some report a reduction in only subtype IIb fibres (Sullivan *et al.*, 1990) while others describe a reduction in the size of both (IIa and IIb) subtypes (Mancini *et al.*, 1989) (Figure 1.3). Gender may play an important role given that female-related skeletal muscle phenotype in patients with moderate CHF is associated with decreased cross-sectional area in both type I and type II fibres but with unexpectedly normal proportion of type I fibres (Tyni-Lenne *et al.*, 1999). An increased in percentage of type IIc fibres, extremely rare in normal subjects, has been also observed (Mancini *et al.*, 1989), which may represent a population of transitional fibres shifting from type I or IIa to type IIb. Despite these alterations in muscle composition and fibre size Mancini *et al.* (1989) noted that the percentage of the total muscle area occupied by each fibre type was unchanged. This apparent compensatory hypertrophy was not accompanied by an associate increase in capillary proliferation resulting in a reduction in capillaries per unit volume of muscle (Drexler *et al.*, 1992). This relative lack of capillary density in oxidative muscle fibre may impair oxidative metabolism and muscle performance. On the contrary, Mancini *et al.* (1989) described no change in capillary number per fibre and Sullivan *et al.* (1990) no change in the ratio of capillaries to cross-sectional fibre area.

Mitochondrial oxidative enzyme activity is also reduced in patients (ß-hydroxyacyl CoA dehydrogenase) and rats (citrate synthase) with left ventricular dysfunction (Mancini *et al.*, 1989; Thompson *et al.*, 1994). The reduction in enzymes involved in ß-oxidation of fatty acids could potentially force higher than normal use of carbohydrates during prolonged exercise, which, in turn, could augment lactate accumulation and reduce endurance because carbohydrate stores are much smaller than fat stores. Discrepancy characterises the findings regarding the activity of enzymes involved in oxidative phosphorylation such as citrate synthase and succinate dehydrogenase in patients with CHF (Mancini *et al.*, 1989; Sullivan *et al.*, 1990); this can be partially explained by mitochondrial volume density measurements obtained in patients which overlap the normal range to a large extent. Recent investigations demonstrate changes in skeletal muscle mRNA expression for oxidative enzymes and myosin heavy chain phenotypes (Simonini *et al.*, 1996; Vescovo *et al.*, 1996). Moreover, in experimental heart failure the phenomenon of apoptosis involves skeletal muscle myofibres, leading to muscle atrophy, and endothelial cells either producing an imbalance in myofibres nutrition (associated with preferential synthesis of fast anaerobic myosin) or inducing myofibres death (Vescovo *et al.*, 1998c). These changes are closely related to the haemodynamic severity of heart failure and not with the level of activity in human and experimental studies, suggesting that the skeletal muscle abnormalities are primary to heart failure and not caused by disuse atrophy. The concept has been, also, put

forward that skeletal muscle changes in patients with idiopathic dilated cardiomyopathy may reflect a generalised myopathic process affecting both the cardiac and the skeletal muscle (Dunnigan *et al.*, 1987; Benditt *et al.*, 1989). Other investigators consider this speculation unlikely since it has been based on qualitative ultrastructural data in a limited number of patients and divergent morphological abnormalities of upper arm skeletal muscle and because these abnormalities (i.e. type II fibre atrophy) were found primarily in patients with symptoms of heart failure and not in patients with asymptomatic left ventricular dysfunction presenting with ventricular arrhythmias (Mancini *et al.*, 1989).

Analysis of the cytochrome *c* oxidase-positive surface mitochondria densities reveals that the total cytochrome *c* oxidase concentration per volume of muscle is reduced, indicating that the oxidative capacity of skeletal muscle is reduced across all fibre types in advanced heart failure. It appears, therefore, more likely that the reduced oxidative capacity of skeletal muscle in CHF is attributed to a general adaptation within all fibre types, resulting in an altered histochemical fibre type pattern caused by changes in the oxidative capacity within each fibre type (Drexler *et al.*, 1992). This evaluation refers to a large cohort of patients with a wide range of functional impairement and is compatible with data from ^{31}phosphorus magnetic resonance spectroscopy (^{31}P MRS) or the biochemical analysis of skeletal muscle observed in a small number of severely ill patients (Mancini *et al.*, 1989; Sullivan *et al.*, 1990). The reduction in the number and size of the mitochondria as well as the relative reduction in capillary density in oxidative muscle fibre would obviously impair skeletal muscle oxidative metabolism and performance and recent studies have attempted to correlate these intrinsic skeletal muscle abnormalities with exercise intolerance seen in CHF (see exercise intolerance section).

Skeletal muscle chemical composition has been, also, evaluated by needle biopsy techniques. Sullivan *et al.* (1990) and Mancini *et al.* (1989) found no abnormalities of protein or myoglobin content and no difference in total creatinine, creatinine phosphate, adenosine triphosphate or diphosphate when compared with control normal subjects. They did, however, reported reduced glycogen levels associated with normal levels of glycolytic intermediates and lactate but with increased pyruvate, indicating an enhanced level of glycolysis in patients with CHF.

III. Metabolism

The first evidence that skeletal muscle metabolic abnormalities develop in patients with CHF came from ^{31}P MRS studies. Analysis of the spectra allows the non-invasive evaluation of the relative concentrations of inorganic phosphate (Pi), phosphocreatine (PCr) and the α, β and γ components of ATP in intact tissues. The Pi/PCr ratio correlates closely with adenosine diphosphate (ADP) concentration, which is the key stimulant of mitochondrial oxidative phosphorylation during exercise. The separation between the Pi and PCr signals is influenced by pH and therefore the phosphate spectra can also be used to estimate intramuscular pH. By monitoring, therefore, changes in Pi/PCr ratio at different workloads,

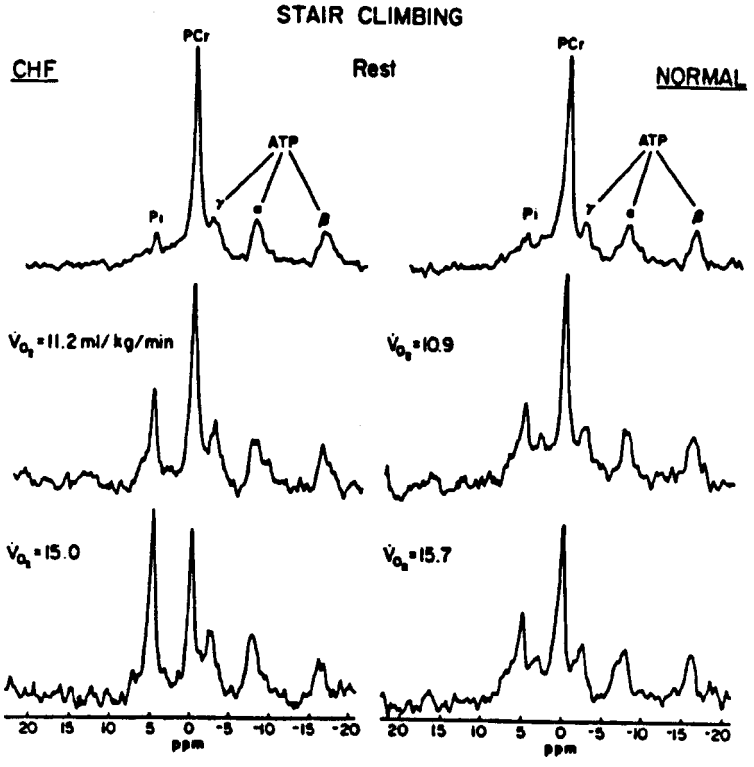

Figure 1.4. Phosphorus-31 nuclear magnetic resonance spectra obtained in a normal subject and in a patient with chronic heart failure (CHF). The rest spectrum and spectra accumulated over two different levels of exercise are provided. As opposed to resting spectrum, the inorganic phosphate (Pi) peak in the patient with heart failure increased more and the phosphocreatine (PCr) peak decreased more than in the normal subject, with both peaks reaching approximately the same height (adapted from Wilson and Mancini, 1993b).

alterations in the control of oxidative phosphorylation can be detected, and changes in muscle pH during exercise, changes in glycolytic activity can be evaluated.

Using ^{31}P MRS Wilson *et al.* (1985c) were the first to report that muscle fatigue in patients with CHF was associated with twice as rapid an increase in Pi/PCr slope, indicative of a greater PCr depletion, and increase in Pi during exercise (Figure 1.4). The slope of the relatioship between the Pi/PCr ratio and power output, an index of oxidative metabolism reflecting the degree of PCr depletion, was steeper in patients with CHF, suggesting an impaired skeletal muscle oxidative capacity. This PCr depletion was associated with a more pronounced decrease in intracellular pH than was noted in the normal subjects performing comparable work levels, though intracellular pH at peak exercise was similar in both groups. This decrease in intracellular pH could be partially responsible for the excessive sympathetic activation during exercise in patients with CHF, because it has been demonstrated that sympathetic stimulation is triggered by alterations in local muscle or circulating blood pH (Victor *et al.*, 1988; Goldsmith *et al.*, 1990). Using slightly different MRS methods and forearm exercise protocols Massie *et al.* (1987b) extended studies on skeletal muscle metabolic properties. Compared with control subjects, these studies

revealed greater PCr utilisation [expressed by the ratio PCr/(PCr+Pi)] and disproportionately greater acidification (in relation to PCr depletion) in patients, suggesting excessive dependence on glycolytic metabolism and offering an additional explanation for muscle fatigue. They were the first to observe delayed PCr resynthesis during the recovery period, indicating an impaired oxidative phosphorylation. The rate of recovery of PCr following exercise represents an important measure of oxidative metabolism, since it is independent of work load and muscle mass (Mancini *et al.*, 1992b). Extending these observations to weight bearing muscles Mancini *et al.* (1988 and 1992b) studied calf muscle. As with the forearm muscle studies, the patients with CHF exhibited greater increases in the Pi/PCr ratio than did the normal subjects. The degree of pH change was related to the strenuousness of exercise. pH alterations were less marked than in forearm studies at low level of exercise, possibly because the muscle contained more type I fibres, whereas at the highest level of exercise patients exhibited a significant decrease in muscle pH compared with normal subjects.

An early onset of lactate production during exercise has been recognised for many years in patients with heart failure observing changes in femoral venous, mixed venous and arterial lactate (Wilson *et al.*, 1984a; Sullivan *et al.*, 1989a). Excessive early lactic acid release could occur as a result of reduced oxygen delivery (due either to impaired cardiac output response to exercise or to attenuated local vasodilation) or as a result of an impaired ability of exercising muscle to utilise oxygen. Although reduced nutritive flow could explain enhanced lactate release (Wilson *et al.*, 1984b), abnormal lactate response to exercise is, also, observed in a subgroup of CHF patients with normal leg blood flow and relatively normal leg arterio-venous oxygen differences during exercise (Wilson *et al.*, 1993a). Furthermore, acute improvement in leg blood flow by infusing hydralazine or dobutamine is not associated with favourable changes in lactate production and oxygen extraction as well as in exercise tolerance, indicating that the increased oxygen supply as a consequence of increased flow is not utilised by the exercising muscle (Wilson *et al.*, 1983a and 1984a). Abnormal, therefore, lactate release in CHF is not simply a result of reduced muscle perfusion but should be, also, attributed to intrinsic skeletal muscle metabolic abnormalities. Katz *et al.* (1993) suggested systemic lactate metabolism as a more accurate index of the metabolic state of exercising muscle (compared with blood lactate) by infusing radiolabeled lactate, which enabled them to calculate lactate turnover and the rate of lactate clearance. Thus, they showed a marked increase in systemic lactate metabolism even at sub-maximal exercise in patients with CHF despite the lack of change in blood (femoral venous or arterial) lactate.

The aetiology underlying skeletal muscle metabolic abnormalities is unclear and studies have attempted to establish the pathophysiology of these abnormalities:

1. *Muscle Atrophy.*

Skeletal muscle atrophy is a frequent finding in CHF (Mancini *et al.*, 1992b) and it has been suggested that the alterations in muscle metabolism could be secondary to atrophy (Buller *et al.*, 1991) or to alteration in the proportion of glycolytic to aerobic fibres within the

muscle. It appears that muscle atrophy is not accompanied by a generalised loss of total body weight and fat stores, thus reducing the skeletal muscle available to mobilise the body, subjecting each fibre to an increased load and in turn producing a greater change in the Pi/PCr ratio and in muscle pH. Atrophy, however, seems to contribute only modestly to both the reduced exercise capacity and the altered muscle metabolism (Mancini *et al.*, 1992b). Moreover, these metabolic changes are still prominent when the workload performed is partially corrected for muscle bulk by comparing findings at 30-70% of each person's maximal workload.

2. *Neurophysiological Abnormalities.*

Muscle activation is not maximal in patients with CHF during exercise; the degree of inactivation, however, is not greater than in sedentary age-matched control subjects. Also, skeletal muscle metabolic changes, resulting in accelerated muscle fatigue, are not caused by abnormal central motor drive or impaired neuromuscular junction transmission (Minotti *et al.*, 1992b). On the contrary, changes in skeletal muscle energy metabolism (PCr resynthesis rate) is accompanied by down-regulation of muscle ß-adrenoceptors, a characteristic reversible after ß-blocker therapy (Michel *et al.*, 1998).

3. *Skeletal Muscle Ischaemia.*

No significant difference was found between plethysmographic forearm blood flows at equivalent workloads in patients with CHF and normal subjects despite the presence of significant intrinsic metabolic abnormalities in the patients during forearm exercise (Wiener *et al.*, 1986). During an identical exercise protocol Massie *et al.* (1987a) showed comparable results, suggesting that the altered metabolic responses of patients with CHF are not due to muscle underperfusion. To further establish this finding, Massie *et al.* (1988) compared forearm metabolic responses to ischaemic exercise (when flow was not present) in patients with CHF and normal controls and observed that even when the muscle was made ischaemic, patients demonstrated lower intracellular pH, greater increases in Pi and enhanced utilisation of PCr. Similarly, other investigators examining muscle function in the knee extensors (Minotti *et al.*, 1991b) or in the adductor pollicis (Buller *et al.*, 1991) demonstrated that muscle endurance was impaired in patients with CHF who exercised when blood flow was occluded. Taken together these observations indicate that in patients with CHF exercising muscle exhibits abnormal metabolism and function which cannot be explained on the basis of skeletal muscle atrophy, impaired neuromuscular function and inadequate skeletal muscle blood flow alone. These findings document an excessive reliance on glycolytic metabolism and are rather consistent with the presence of intrinsic skeletal muscle metabolic abnormalities in patients with CHF.

4. *Mutations of Mitochondrial DNA.*

Excessive accumulation of free radicals, induced by recurrent episodes of hypoxaemia and reperfusion, may account for intrinsic skeletal muscle metabolic abnormalities in patients with CHF (Corral-Debrinski *et al.*, 1991), given that free radicals are known to mutagenise mitochondrial DNA as well as enzymes involved in oxidative phosphorylation.

IV. The Role of Deconditioning

The underlying cause(s) of muscle dysfunction and intrinsic metabolic abnormalities is uncertain but one potential contributing factor might be inactivity leading to deconditioning, which is known to produce metabolic and functional alterations similar to those observed in CHF. Both conditions are associated with exercise intolerance, wasted skeletal muscle (Holloszy, 1976; Buller *et al.*, 1991) and depleted skeletal muscle oxidative enzymes (Holloszy, 1976; Sullivan *et al.*, 1990).

Patients with CHF are traditionally instructed by physicians to decrease their activity levels; in addition, patients with severe CHF are frequently hospitalised and subjected to long periods of bedrest. Significant improvements with physical training in exercise performance by 15-30% (Sullivan *et al.*, 1988a; Coats *et al.*, 1990), as assessed by exercise tolerance and peak oxygen consumption, provide the best evidence that at least a part of the skeletal muscle metabolic and functional defect, present in patients with CHF, is due to inactivity. Significant, also, improvements with local muscle training in skeletal muscle oxidative capacity (Minotti *et al.*, 1990b; Stratton *et al.*, 1994), as assessed by ^{31}P MRS, without associated changes in forearm muscle size or exercise blood flow and, also, without changes in the endurance or metabolism of the untrained forearm, are compatible with the hypothesis that muscle disuse is responsible, at least partially, for abnormal skeletal muscle metabolic responses to exercise.

The effects of training, however, are non-specific and do not necessarily imply that muscle deconditioning is the underlying cause of the initial metabolic abnormalities (Minotti *et al.*, 1990a). In addition, local muscle detraining does not appear to exclusively explain skeletal muscle abnormalities because muscle dysfunction can occur in muscles not expected to be deconditioned. Muscle endurance was reduced in a small hand muscle (Buller *et al.*, 1991) and impaired in the knee extensors (Minotti *et al.*, 1991b) and in a relatively small postural muscle of the leg (tibialis anterior) that is not used for weight bearing (Minotti *et al.*, 1992b). Furthermore, the hypertrophied type I fibres observed in biopsy studies (Minotti *et al.*, 1993b) are not exclusively confined in deconditioned skeletal muscle. Intrinsic skeletal muscle metabolic abnormalities, therefore, cannot be attributed solely to muscle disuse, are rather specific to CHF and are modified by muscle inactivity in various degrees.

Several other factors also have been implicated in the skeletal muscle functional and metabolic alterations of CHF. Metabolic and/or hormonal derangements that favour catabolism over anabolism may also substantially contribute to skeletal myopathy. Increased release of TNF-a (Levine *et al.*, 1990), abnormalities in the handling of intracellular thyroid hormone, elevated cortisol levels, insulin resistance, as well as caloric-protein malnutrition (Carr *et al.*, 1989) and intestinal malabsorption, with consequent bacterial translocation and endotoxin release causing immune reaction with increased TNF-a production (Anker *et al.*, 1997b), may play a role in producing muscle changes in a subgroup of patients.

V. Skeletal Muscle Mass

Apart from intrinsic skeletal muscle abnormalities a substantial reduction of total skeletal muscle bulk seems to characterise advanced CHF. Despite the marked muscle atrophy observed in cardiac cachexia, it has been only recently recognised that skeletal muscle atrophy can occur early in the course of the syndome, without documented weight loss. It may be related to physical deconditioning or elevated circulating cytokine levels (Levine *et al.*, 1990). Mancini *et al.* (1992b), using creatinine/height index and/or mid arm muscle circumference, demonstrated muscle atrophy in 68% of studied patients which was not accompanied by significant abnormalities in protein synthesis or body fat stores. Using magnetic resonance imaging (MRI) they also confirmed that calf muscle volume, even when normalised for body surface area, is significantly reduced when compared with normal control subjects, a finding repeated by Minotti *et al.* (1993c), who used maximal cross-sectional area of the thigh muscles also determined by MRI. This pattern, therefore, of changes results in maintenance of body weight but loss of skeletal muscle available to mobilise the body. Because less muscle is available, then each fibre is subjected to more workload only able to accept lower total blood flow and will appear metabolically more stressed and, therefore, be more easily fatigued, requiring anaerobic metabolism at an earlier stage of exercise. Although maximal strength was directly proportional to muscle size, muscle endurance was not, indicating that atrophy contributes modestly to the reduced exercise tolerance and impaired skeletal muscle metabolism, given the weak correlations between muscle volume and both peak VO_2 and ^{31}P metabolic abnormalities (Mancini *et al.*, 1992b, Minotti *et al.*, 1993c). Volterrani *et al.* (1994), however, reported a significant correlation between a measure of leg volume (mid-femour quadriceps cross-sectional area) and peak oxygen consumption proving skeletal muscle bulk an important marker of exercise tolerance.

C. Physical Training and Skeletal Muscle in Heart Failure

Physical inactivity results in decreased mitochondrial enzyme activity and physical training has been shown to increase ß-hydroxyacyl CoA dehydrogenase and citrate synthase activity (Holloszy, 1976) in normal subjects. Training is also known to induce an increase of the level of glutamate pyruvate transferase, a mitochondrial-cytoplasmic enzyme which permits the generation of alanine and ketoglutarate from pyruvate and glutamate, thus reducing the formation of lactate during exercise and increasing the pH, providing at the same time more oxaloacetate to the first step of Krebs cycle (Mole *et al.*, 1973; Holloszy, 1976). More recently in normal subjects, training has been associated with improved skeletal muscle oxidative capacity, as assessed by ^{31}P MRS studies. Thus, forearm training led to a higher pH at submaximal workloads (Kent-Braun *et al.*, 1990). A reduction in Pi/PCr at submaximal workloads and an increase in PCr recovery rates have also been found with training, indicative of an improvement in oxidative metabolism (McCully *et al.*, 1989 and 1991).

Recent investigations have established the beneficial role of exercise training on skeletal muscle function and metabolism in patients with CHF. Although remains controversial whether the morphological, histochemical and biochemical alterations as well as functional abnormalities in skeletal muscle represent disuse or specific characteristics associated with CHF, the benefits of training have been well documented. Thus, Sullivan *et al.* (1988a) were able to demonstrate significant improvement in exercise capacity, expressed by peak oxygen consumption, in response to 4-6 months of systemic exercise training in patients with stable CHF. The same investigators showed lessening of the metabolic abnormalities observed before training, expressed by a reduction in both arterial and venous lactate levels. Since the conditioning stimulus was systemic, it is unclear whether the measured changes in the exercising limb, suggesting an improved skeletal muscle response to exercise, are attributable solely to peripheral adaptations. Employing localised (wrist flexor) conditioning in patients with CHF, Minotti *et al.* (1990b) demonstrated improvement in muscle bioenergetics, as assessed by the slope of the regression line relating Pi/PCr to submaximal workloads, and increase in forearm endurance by 260%, effects which are independent of muscle mass, limb blood flow, plasma catecholamines or a central cardiovascular response. Coats *et al.* (1990) confirmed the benefits of training on exercise tolerance and symptoms in patients with moderate to severe CHF by using, for the first time, a randomised, controlled design and home-based exercise regimen. A more recent study demonstrates that the metabolic abnormalities seen in exercising skeletal muscle in patients with CHF can be reversed, at least partially, by local muscle training either during incremental or during endurance exercise (Stratton *et al.*, 1994). The reduction in acidification and PCr utilisation during exercise as well as the increases in maximal rate of mitochondrial ATP synthesis (Q_{max}) and PCr resynthesis rate during recovery indicate an increased capacity for oxidative metabolism and a decreased dependence on glycolysis in the trained skeletal muscles.

Regular (even low intensity) physical training not only increases exercise tolerance and delays anaerobic metabolism during submaximal exercise in patients with CHF but, also, improves oxidative capacity of the exercising muscle by increasing the total volume density of mitochondria and the volume density of cytochrome *c* oxidase-positive mitochondria (Belardinelli *et al.*, 1995; Hambrecht *et al.*, 1995). This significant increase in oxidative capacity of skeletal muscle (vastus lateralis) after endurance training is significantly correlated with the improvement in functional capacity and peak oxygen uptake; patients with severe exercise intolerance and/or severe ventricular dysfunction exhibit the most impressive training-induced changes in oxidative capacity of skeletal muscle and in peak oxygen consumption. In addition, change in oxygen consumption at ventilatory threshold is significantly correlated with change in volume density of cytochrome *c* oxidase-positive mitochondria in skeletal muscle, suggesting that the improved oxidative capacity of the mitochondria may account for the delayed onset of ventilatory threshold after exercise training (Figure 1.5).

Apart from muscle conditioning in the training programmes, what are the other possible explanations of the training-induced improvement in skeletal muscle oxidative capacity?

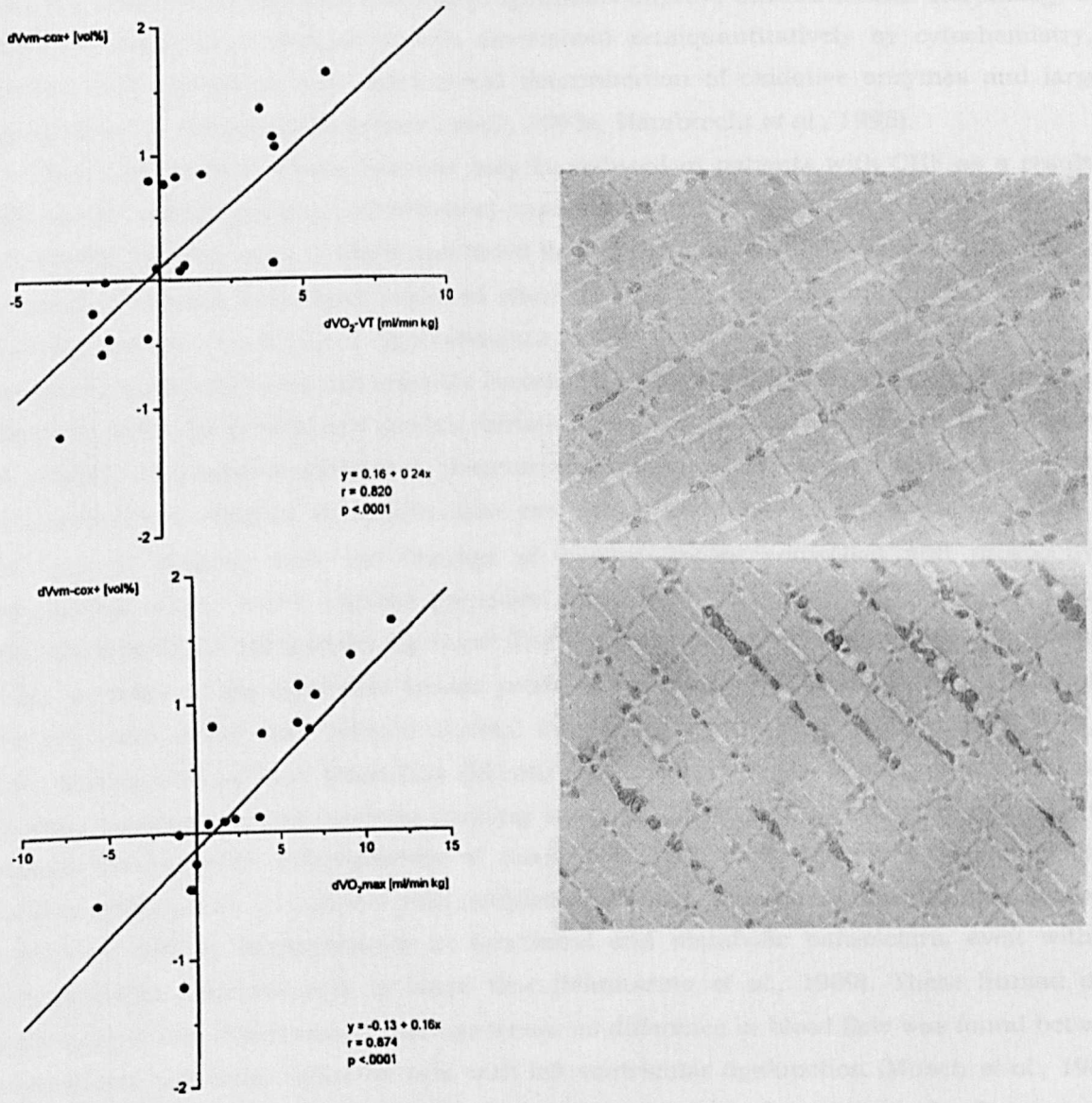

Figure 1.5. Physical training-induced changes in volume density of cytochrome *c* oxidase-positive mitochondria in patients with chronic heart failure. Changes in volume density of cytochrome *c* oxidase-positive mitochondria (dVvm-cox+) correlated with change in oxygen uptake at ventilatory threshold (dVO_2-Vt) (left panel, top) and change in peak oxygen uptake (dVO_2max) (left panel, bottom). Electron micrographs of cytochrome *c* oxidase in a patient with heart failure before (right panel, top) and after (right panel, bottom) 6 months of exercise training. Note the increased enzyme activity within the mitochondria (black stain) after training (adapted from Hambrecht *et al.*, 1995).

The MRS changes observed in patients with CHF after training, could have simply been resulted from performing the same work with more muscle induced by training. Although the possibility of reversing wasting of skeletal muscles by training cannot be excluded, it has been shown that localised skeletal muscle training can produce beneficial ^{31}P MRS responses at submaximal workloads without any associated change in muscle mass (Minotti *et al.*, 1990b). Moreover, the same investigators and more recently Stratton *et al.* (1994) by using recovery characteristics showed significant training-induced improvement in PCr resynthesis rate and in mitochondrial Q_{max}, indices independent of muscle mass. In

addition, ambulatory physical training programmes improve ultrastructural morphology and oxidative capacity of skeletal muscle determined semiquantitatively by cytochemistry, a method well correlated with biochemical determination of oxidative enzymes and largely independent of muscle mass (Minotti *et al.*, 1993a; Hambrecht *et al.*, 1995).

Muscle blood flow during exercise may be reduced in patients with CHF as a result of diminished cardiac output and impaired vasodilatory reserve (Zelis *et al.*, 1974; Wilson *et al.*, 1984b; Sullivan *et al.*, 1989b) and blood flow alterations resulting from an enhancement in capillary density have been observed after physical training (Laughlin *et al.*, 1988). The training induced biochemical improvements could, therefore, be explained by a reduced peripheral resistance with concomitant increase in peak blood flow to the exercising leg and markedly reduced arterial and venous lactate levels that have been reported by Sullivan *et al.* (1988a). The same investigators, however, provide evidence that after training the lactate production was delayed at submaximal exercise independent of leg blood flow and O_2 delivery, in keeping with the findings of a more recent controlled and prospectively randomised study, which showed decreased submaximal blood lactate levels after regular exercise despite an unchanged leg blood flow at submaximal work loads, indicating, also, a delay in onset of leg anaerobic lactate production (Hambrecht *et al.*, 1995). It has, also, recently been noted that reduced skeletal muscle endurance in patients with CHF, is, in part, independent of limb blood flow (Minotti *et al.*, 1991b). Again Minotti *et al.* (1990 a&b) in their localised forearm exercise training study in CHF patients showed improvement in muscle bioenergetics independently of changes in limb blood flow or in the underlying cardiac dysfunction. In patients with peripheral vascular disease, exercise training has been shown to lead to improvements in functional and metabolic parameters, even without demonstrable improvements in blood flow (Mannarino *et al.*, 1989). These human data confirm previous experimental findings where no difference in blood flow was found between trained and sedentary infarcted rats with left ventricular dysfunction (Musch *et al.*, 1986). However, despite these findings, redistribution of blood flow within the leg to more effectively perfuse the exercising muscles cannot be excluded (Mackie *et al.*, 1983). In addition, although exercise of small muscle groups may not be limited by blood flow, exercise of large muscle groups, which may be more responsible for exercise intolerance in CHF, may be limited by blood flow. Finally, the corrections of muscle metabolic changes after training could also be explained by the differences in blood flow distribution towards glycolytic muscles prior to training (Drexler *et al.*, 1987) and towards oxidative muscles after training (Musch *et al.*, 1992a). Although training-induced improvements in metabolic responses to exercise are compatible with the hypothesis that deconditioning can cause part of the skeletal muscle metabolic defect in CHF, one cannot exclude the possibility that patients may continue to have a primary defect due to oxygen delivery and utilisation and the training effect was non-specific.

Improvements in oxidative metabolism with exercise training could be due to an increase in mitochondrial number or an increase in the oxidative capacity of existing mitochondria, in keeping with animal and human data in normal subjects (Holloszy, 1967; Gollnick *et al.*,

1969 and 1973) suggesting increase in mitochondrial content and oxidative enzymes with endurance training. Recent studies have focused on the effects of training programmes on skeletal muscle oxidative capacity in patients with moderate to severe CHF and found significant increases in the total volume density of mitochondria and volume density of cytochrome *c* oxidase-positive mitochondria independently from muscle mass or peripheral blood flow (Hambrecht *et al.*, 1995). Even more recently, evidence is provided that regular physical exercise improves both basal endothelial NO formation and agonist-mediated endothelium-dependent vasodilation of the skeletal muscle microvasculature in patients with CHF by increasing expression of endothelial NO synthase and preventing the production of vasoconstrictor prostanoids and free radicals (Varin *et al.*, 1999). Training-induced improvement in endothelium dysfunction is associated with a significant increase in exercise performance (Hambrecht *et al.*, 1998). Restoration, at least partial, of endothelial function with physical training may contribute to the redistribution of skeletal muscle blood flow with a preferential supply to oxidative muscles during submaximal exercise, thus explaining the increase in oxidative enzyme capacity of the working skeletal muscle observed in patients with CHF, which is closely related to the improved exercise tolerance after physical training (Hambrecht *et al.*, 1995 and 1997).

The contribution of peripheral neural adjustments to the decreased PCr depletion during exercise and improved oxidative ATP synthesis (expressed by Q_{max}) after training cannot be excluded. These may be related to either alterations in recruitment of motorneurons with endurance training or increased sensitivity of β_2 muscle metabolic receptors compatible with the reduced sympathetic activity found with training (Coats *et al.*, 1990).

Whatever the potential mediator of the positive effects of exercise training on the exercising muscle, the resolution of skeletal muscle abnormalities may be responsible for the reduced activity of the exaggerated muscle ergoreflex, thereby improving the abnormal responses to exercise (heightened sympathetic, vasoconstrictor and ventilatory drives) characteristic of heart failure (Piepoli *et al.*, 1996).

D. Exercise Intolerance in Heart Failure - The Role of Skeletal Muscle

Investigators have sought to define the mechanisms responsible for exercise intolerance in CHF. For over a century, exertional dyspnoea was attributed to exercise-induced increases in pulmonary artery and wedge pressures and exertional fatigue to an inadequate cardiac output due to impairment in ventricular performance. Over the past 15 years it has become clear that the link between cardiac dysfunction and exercise capacity in heart failure is far more complex. The inability of haemodynamics to explain the generation of symptoms in CHF tests (Franciosa *et al.*, 1981; Gibbs *et al.*, 1990; Sinoway *et al.*, 1988) has shifted the emphasis of attention away from the heart and towards non-haemodynamic peripheral factors and especially towards the exercising muscle for a more detailed understanding of the pathogenesis of exertional dyspnoea and muscle fatigue.

Observations that acute alterations of haemodynamic parameters did not alter exertional symptoms provided the first evidence that non-circulatory factors contribute to exercise intolerance in CHF. Thus, after pharmacological manipulations acute reductions in the pulmonary wedge pressure during exercise had no effect on ventilatory responses or the perception of dyspnoea (Fink *et al.*, 1986). Moreover, excessive ventilatory levels during exercise had no relation to the resting or exercise-induced pulmonary wedge pressure (Sullivan *et al.*, 1988b). Also, a variety of pharmacological interventions, including ACE inhibitors, *a*-blockers, hydralazine and phosphodiesterase inhibitors, have been tested to acutely increase cardiac output and estimate the exercise tolerance before and after the intervention in patients with CHF (Wilson *et al.*, 1983a, 1984a and 1985a; Maskin *et al.*, 1983; Drexler *et al.*, 1989). Although such interventions enhanced both cardiac output and leg blood flow, patients manifested no improvement in their exercise capacity, sensation of leg fatigue or leg lactate release. Conversely, long-term ACE inhibition improved systemic oxygen uptake through, probably, peripheral (mainly vascular) mechanisms, by reversing the inability of peripheral vessels to dilate adequately during exercise, by improving oxygen utilisation of the working muscle (Drexler *et al.*, 1989) and by reversing, partially, the ultrastructural abnormalities of skeletal muscle (Drexler *et al.*, 1992) in patients with CHF. A reshift of the contractile proteins (MHCs, namely MHC1, slow aerobic; MHC2a, fast oxidative; and MHC2b, fast glycolytic) toward the slow, more fatigue-resistant, oxidative fibres has been shown after treatment with ACE inhibitors and angiotensin II antagonists in CHF (Vescovo *et al.*, 1998a). Since the magnitude of slow MHC1 changes correlates with the net peak oxygen consumption gain, it can be argued that the increased exercise capacity could be, partially, explained by the favourable biochemical changes occuring in the skeletal muscle after 6 months' treatment. Recent experimental and clinical data (Hornig *et al.*, 1998) support the concept that ACE inhibitors improve vascular function by increasing the availability of bradykinin, thereby enhancing the endothelial release of NO.

Although restrictions in peak blood flow to exercising muscle have been shown in CHF (Wilson *et al.*, 1984b; Sullivan *et al.*, 1989b) it is not clear to what extent this is due to impaired endothelial dependent vasodilatory capacity, to persistent vasoconstrictor activity or to a reduction in either muscle capillarity and/or muscle volume. However, a substantial number of patients with muscle fatigue and lactate abnormalities exhibit normal leg flow responses to exercise (Wilson *et al.*, 1993a), not necessarily proving but strongly suggesting that muscle rather than flow abnormalities are limiting exercise in heart failure. Even in patients where the total peak blood flow to an exercising limb is reduced, where the flow is expressed per unit muscle volume (such as by plethysmography) the results are contradictory, indicating that a deficiency in the total muscle bulk may be responsible, at least partially, for the reduced blood flow in CHF (Coats *et al.*, 1994). Further evidence from ^{31}P MRS studies makes a primary reduction in blood flow unlikely to be the major cause of exercise limitation in CHF. Thus, several investigators have examined forearm and calf metabolic responses to local limb exercise and observed more rapid decrease in intracellular muscle pH and PCr concentration in patients with CHF compared with normal subjects

(Wilson *et al.*, 1985c; Wiener *et al.*, 1986; Massie *et al.*, 1987b; Mancini *et al.*, 1989). Despite these muscle metabolic abnormalities, when blood flow to exercising limb was measured there was no evidence of inadequate muscle perfusion, implying skeletal myopathy in CHF. These non-invasive findings were subsequently confirmed by biopsy studies. Several groups independently reported ultrastructural (shifts from slow-twitch type I to more fatigable fast-twitch type II fibres and atrophy of the fast-twitch type II fibres) and cytochemical (reduced mitochondrial size and levels of oxidative enzymes) alterations in the leg muscles of patients with CHF (Mancini *et al.*, 1989 and 1992; Sullivan *et al.*, 1990; Drexler *et al.*, 1992). In addition, impaired quadriceps muscle endurance in patients with CHF was independent of limb blood flow (Minotti *et al.*, 1991b). Finally, in moderate-to severe CHF patients significant muscular cellular metabolic alterations are already present at rest and increase during light daily activity, independent of peripheral haemodynamic responses to exercise. This decrease in the efficiency of muscle metabolic processes may, also, preclude tolerance of heavier activities (Opasich *et al.*, 1997). The importance of restricted blood flow on exercise capacity in CHF is progressively increasing with the development of cardiac cachexia (Anker *et al.*, 1997c). This is in keeping with a recent study showing that exercise performance and local circulatory dysfunction are decreased in parallel in patients with severe CHF (Toussaint *et al.*, 1998).

Similar structural and functional abnormalities have been observed in respiratory muscle of patients with CHF. A variety of histological abnormalities in the diaphragmatic muscle has been reported (Lindsay *et al.*, 1992a) and reductions in maximal inspiratory and expiratory pressures, consistent with respiratory muscle weakness, have been desribed (Hammond *et al.*, 1990; McParland *et al.*, 1992) in patients with CHF. Also, the work performed by the diaphragm in patients with CHF during exercise is increased and the accessory respiratory muscles are deoxygenated (Mancini *et al.*, 1992a).

Abnormalities of skeletal muscle histology (Lipkin *et al.*, 1988), capillary density (Duscha *et al.*, 1999), mitochondria (Drexler *et al.*, 1992), oxidative enzyme activity (Sullivan *et al.*, 1990) and high energy phosphate handling (Massie *et al.*, 1987b) have all been described in CHF, as has early muscle fatigue (Buller *et al.*, 1991). Any one of these abnormalities could explain exertional fatigue in patients with CHF and undoubtedly a combination of factors is operative in some patients. Thus, a significant correlation has been shown (Mancini *et al.*, 1989) between peak oxygen consumption and both % type I oxidative (positive correlation) and % type IIb glycolytic (negative correlation) fibres. A correlation has also been observed between the percent distribution of the 3 myosin heavy chains (MHCs) in the gastrocnemius, the severity of the heart failure syndrome and exercise tolerance expressed by peak oxygen uptake and ventilatory threshold (Vescovo *et al.*, 1998b). Recent investigations demonstrate that significant decreases in microvascular (capillary) density may be partially responsible for impaired exercise capacity in patients with mild to moderate CHF without other major intrinsic (histologic and biochemical) abnormalities in skeletal muscle (Duscha *et al.*, 1999). In addition, mitochondrial volume density and surface density of mitochondrial cristae are significantly related to peak exercise oxygen uptake and to

oxygen uptake at anaerobic threshold (Drexler *et al.*, 1992). It has been recently shown that mitochondrial creatine kinase (CK), a key enzyme for rapid energy transfer from mitochondria to cytosol, is significantly reduced, whereas the expression of inducible NO synthase (iNOS) is increased in the skeletal muscle of patients with CHF (Hambrecht *et al.*, 1999). The inverse correlation between mitochondrial CK and iNOS expression indicates that NO produced by iNOS attenuates mitochondrial energy transfer, thus accounting for the early muscular fatigue and exercise intolerance in CHF. Muscle metabolic capacity, evaluated as the slope of PCr decrease in relation to increasing workload, is correlated with peak oxygen consumption during maximal systemic exercise in patients with CHF (Okita *et al.*, 1998). Also, the degree of muscle dysfunction (assessed by the force generated per muscle extension) is correlated with the peak systemic oxygen consumption (Buller *et al.*, 1991; Minotti *et al.*, 1991a). Recent reports suggest that the resting variables that best predict exercise performance in CHF are measures of skeletal muscle function (muscle strength) and bulk (muscle cross sectional area), indicating that some of the qualitative alterations may also be a reflection of skeletal muscle wasting (Volterrani *et al.*, 1994). Loss of quadriceps bulk seems to represent an important quantitative abnormality contributing to the progressive skeletal muscle weakness which is associated with increasing exercise limitation in patients with CHF (Harrington *et al.*, 1997). Metabolic or hormonal derangements, including release of TNF-a (Packer, 1995), insulin resistance (Swan *et al.*, 1994), abnormal handling of intracellular thyroid hormone (Anker *et al.*, 1997a) as well as intestinal malabsorption (Anker *et al.*, 1997b), that favour catabolism over anabolism may also contribute to myopathy. Similarities between muscle abnormalities associated with CHF and those observed in conditions with chronic inactivity (deconditioning) as well as research on the beneficial effects of physical training on exercise performance have suggested that inactivity may also represent an important mechanism of skeletal myopathy in CHF. Whatever the underlying mechanism skeletal muscle fatigue is more likely to reside in the intracellular control of Ca^{++} release and reuptake from the sarcoplasmic reticulum (Lunde *et al.*, 1998). The decreased sarcoplasmic reticulum Ca^{++}-ATPase protein (SERCA) expression may underlie the impaired Ca^{++} handling characterising skeletal muscles and contribute to contractile abnormalities related to excitation-contraction coupling function in CHF (Peters *et al.*, 1997).

The frequent coexistence of muscle fatigue and dyspnoea and the delay in resolution of both symptoms following significant haemodynamic improvement (i.e. heart transpalntation) raise the possibility that structural skeletal muscle changes may be involved in the pathogenesis of fatigue as well as dyspnoea in CHF. Dyspnoea and increased ventilation could also be attributed to abnormalities in respiratory musculature similar to those described in skeletal muscle (Lindsay *et al.*, 1992a; McParland *et al.*, 1992; Mancini *et al.*, 1992a), although other investigators were unable to find a relationship between the abnormal ventilatory findings and exercise performance (Wilson *et al.*, 1992). An alternative explanation has been recently proposed by some investigators who believe that skeletal

muscle signals contribute directly to the perception of both muscle fatigue and dyspnoea via an exaggerated neural signal from the muscle (skeletal muscle ergoreflex). According to this "muscle hypothesis" skeletal and respiratory myopathy sensitises muscle "ergoreceptors" with an enhanced contribution to the autonomic, haemodynamic and respiratory responses to exercise in patients with CHF (Piepoli *et al.*, 1996). These observations serve to emphasise that exercise intolerance in CHF must be viewed as a product of both skeletal (and respiratory) muscle and cardiovascular dysfunction; improving skeletal and respiratory muscle characteristics may be just as important as altering the cardiovascular system.

The hypothesis that exertional symptoms in CHF could be improved by altering skeletal muscle performance was initially tested by using whole-body exercise training. Sullivan *et al.* (1989a) in a standard cardiac rehabilitation programme and Coats *et al.* (1992) in a randomised crossover study (home-based bicycle exercise programme) noted that this intervention significantly improved maximal exercise capacity and patient-scored symptoms during normal daily activities, reduced skeletal muscle lactate release during exercise and ventilatory responses to exercise. No significant changes in haemodynamic responses to exercise were found. The significant role of skeletal muscle structural and metabolic abnormalities in generating and/or perpetuating the cardinal symptoms of heart failure is confirmed by recent studies where exercise training-induced increase in the oxidative capacity of skeletal muscle, assessed by change in volume density of cytochrome *c* oxidase-positive mitochondria (Belardinelli *et al.*, 1995; Hambrecht *et al.*, 1995 and 1997), is closely linked to improved functional capacity and peak oxygen uptake.

Mancini *et al.* (1995) sought to apply the same approach to the respiratory muscles and, in a carefully designed respiratory muscle training regimen, they managed to demonstrate significantly increased maximal inspiratory and expiratory pressure, maximal voluntary ventilation and improved peak exercise VO_2. The beneficial effect on respiratory muscle strength, expressed by increases (of almost 50% on average) of maximum sustainable ventilatory capacity, was associated with dramatic reduction in the sensation of dyspnoea.

Recent observations suggest that physical training not only improved the exercise capacity of the trained forearm but also partially reversed the exaggerated ergoreflex activity, thereby improving the enhanced sympathetic, vasoconstrictor and ventilatory drives characteristic of CHF (Piepoli *et al.*, 1996). Resolution of both muscle fatigue and dyspnoea and the excessive reflex responses to exercise in CHF may, therefore, depend on resolution of the abnormal function of skeletal muscle.

In conclusion, the benefits of exercise training on skeletal muscle structure and function and the parallel enhancement in exercise tolerance associated with reduction in dyspnoea and ventilatory abnormalities suggest that by looking beyond the conventional haemodynamic remedies and focusing on specific muscle treatments may help in the management of CHF. The sustained improvement in functional capacity and quality of life, achieved with physical training, seems to translate not only into a lower rate of hospital readmission for cardiac insufficiency but also, for the first time, into a lower mortality rate (Belardinelli *et al.*, 1999).

E. Neurohormonal Activation in Chronic Heart Failure

Early in the progression of heart failure from mild asymptomatic left ventricular dysfunction to the full clinical expression of the syndrome of CHF there is a neurohormonal excitation, characterised by activation of the sympathetic nervous system associated with a parasympathetic withdrawal, activation of the arginine-vasopressin system, the renin-angiotensin-aldosterone (RAA) system, as well as the counteracting atrial natriuretic peptides (Francis *et al.*, 1984; Swedberg *et al.*, 1990). Neuroendocrine activation appears to precede overtly symptomatic heart failure with significant increases in plasma levels of NA, atrial natriuretic peptide (ANP) and arginine-vasopressin (AVP) and may, therefore, contribute to its development. As overt heart failure ensues and diuretics are added, these neurohormonal changes are further enhanced and plasma renin activity becomes also abnormal (elevated) (Francis *et al.*, 1990a). Similar findings have been observed in the pacing animal model of heart failure (Armstrong *et al.*, 1986).

A myocardial insult (mainly myocardial ischaemia or cardiomyopathy) is followed by activation of vasoconstrictor, via enhanced sympathetic tone, and vasodilator forces to restore cardiac function towards that existed before injury at minimum energetic cost. Hence, atrial stretch initially stimulates atrial baroreceptors (Hirsch *et al.*, 1987) that inhibit sympathetic outflow from the vasomotor centre and leads to a secretion ANP (Floras, 1990; Kimura *et al.*, 1990) which, apart from direct vasodilator and natriuretic effects inhibits the release of NA and the actions of this neurotransmitter on peripheral blood vessels. Prolonged atrial distension, however, lead to impairment of baroreceptor sensitivity (Hirsch *et al.*, 1987; Ellenbogen *et al.*, 1989) and depletion of natriuretic peptides (Moe *et al.*, 1991). Loss of these stress lowering compensatory mechanisms heralds the beginning of heart failure, where sympathetic nervous system becomes persistently activated. The sympathetic nervous system is activated early in the disease process (when compensatory ventricular dilatation takes place), whereas RAA system is usually triggered once symptoms develop (especially after the administration of diuretics) and AVP is released in the more advanced stages of the disease when perfusion pressure is threatened (Creager *et al.*, 1986).

i. Adrenergic Nervous System

Chronic activation of the adrenergic system, decreased parasympathetic activity and impaired arterial baroreflex sensitivity have all been described as part of the syndrome of CHF (Eckberg *et al.*, 1971; Cohn *et al.*, 1984; Leimbach *et al.*, 1986; Ferguson *et al.*, 1992). The sympathetic nervous system is normally modified by a) inhibitory influences from the cardiopulmonary (sensitive to chemical and/or mechanical stimuli) and aortic (sensitive to mechanical stimuli) baroreceptors, which are tonically active, b) excitatory afferents, which are activated under certain circumstances such as exercise-induced ischaemia (muscle metaboreceptors), hypoxaemia (peripheral chemoreceptors) or ischaemic metabolites (cardiac sympathetic afferents), and c) centrally acting neuromodulators, which may be

either inhibitory (opiates, nitric oxide) or excitatory (ouabainlike activity, angiotensin II, NA) (Middlekauff, 1997) (Figure 1.6).

1. *Baroreflex Dysfunction*

There is no clear mechanism to explain either the activation of the sympathetic nervous system in asymptomatic left ventricular dysfunction or the persistence of this activation and progression in the chronic syndrome. Afferent signals from the cardiac chambers and great vessels, when stimulated, inhibit the cardiovascular centres in the brain, resulting in reduced sympathetic outflow from the central nervous system. Abnormalities of the baroreceptor function lead to withdrawal of the sympatho-inhibitory effects of the cardiac and arterial baroreflexes on the vasomotor centre in the central nervous system. The effect of cardiopulmonary and arterial baroreceptor stimulation on corresponding afferent neural activity is attenuated in experimental models of heart failure. Thus, several studies (Greenberg *et al.*, 1973; Zucker *et al.*, 1977) suggested decreased atrial receptor activity and sensitivity in dogs with heart failure compared with control dogs, possibly attributed to both decreased atrial compliance and abnormalities in atrial receptor morphology, which are reversible following reversal of heart failure. This resetting of atrial receptors may be responsible for the inappropriately high plasma antidiuretic hormone levels in CHF and may contribute to clinical characteristics of advanced CHF, including peripheral oedema, ascites, hyponatraemia and renal vasoconstriction (Riegger *et al.*, 1982). Decreased, also, ventricular compliance and reset in the pressure-discharge relationship has been hypothesised to underlie the blunted ability of ventricular vagal afferents to respond to mechanical stimuli, such as changes in ventricular pressure, in animal models of heart failure (Dibner-Dunlap *et al.*, 1992). Thus, a blunted suppresion of efferent renal sympathetic nerve activity during volume expansion has been found in sinoaortic denervated dogs with pacing-induced low-output heart failure, attributing to abnormalities in cardiopulmonary baroreflexes. Similarly, in dogs with experimental heart failure, carotid artery occlusion or carotid sinus pressure elicit a blunted reflex increase in heart rate, blood pressure and mesenteric and renal vascular resistance (Higgins *et al.*, 1972; White, 1981). Augmented carotid baroreceptor sinus Na^+-K^+ ATPase activity has been described in dogs with heart failure (Wang *et al.*, 1990). The selective perfusion of the carotid sinus with ouabain augments baroreceptor sensitivity in dogs with pacing-induced heart failure, providing promising evidence of a reversible ionic mechanism of baroreflex dysfunction in this model of heart failure. Digitalis glycosides can correct, at least partially, the blunted baroreceptor responsiveness in patients with CHF by acting directly on one or more components of the baroreflex pathway rather than by improving the haemodynamic function (Ferguson *et al.*, 1984). Comparable baroreflex function has been observed in humans with CHF. Low levels of lower-body negative pressure, maneuver enabling characterisation of the reflex responses to cardiopulmonary baroreceptor unloading, causes significantly less forearm vasoconstriction in patients with CHF than in healthy subjects (Ferguson *et al.*, 1984; Hirsch *et al.*, 1989;

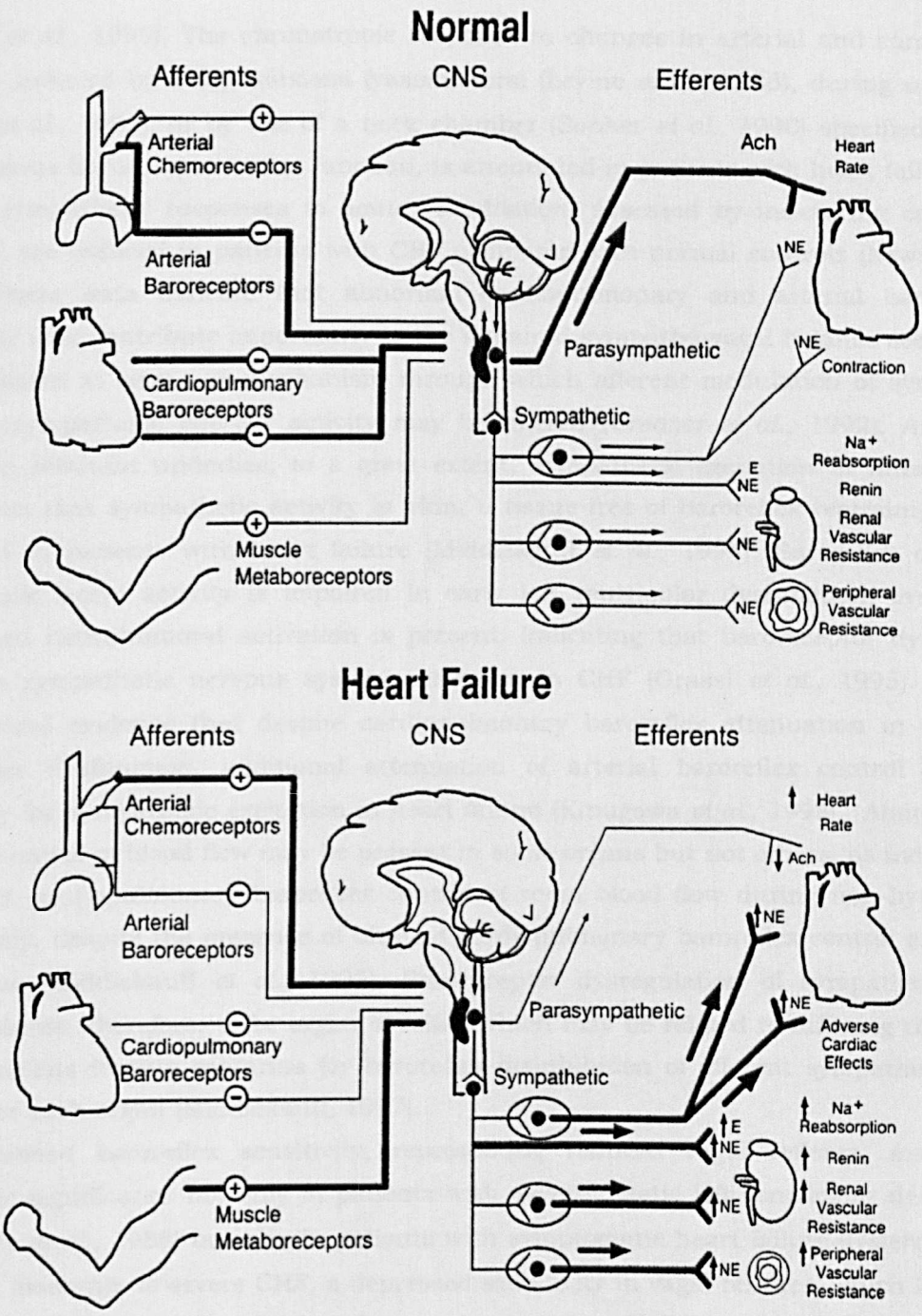

Figure 1.6. Generalised sympathetic activation and parasympathetic withdrawal in heart failure: inhibitory and excitatory influences on brainstem vasomotor neurons. Afferent inputs from carotid sinus and aortic arch "arterial high pressure" and the cardiopulmonary "low pressure" baroreceptors are the principal inhibitory influences on sympathetic outflow; discharge from arterial chemoreceptors and muscle "metaboreceptors" are the major excitatory inputs. Parasympathetic control of heart rate is also influenced by arterial baroreceptor afferent input. The integrated response to these competing influences in normal subjects may be characterised as a relatively low sympathetic discharge associated with low arterial catecholamines and high heart rate variability whereas as heart failure progresses the net response includes a generalised enhancement in sympathetic nerve traffic, blunted parasympathetic control of heart rate with relatively low heart rate variability and impairment of the reflex sympathetic regulation of vascular resistance. Ach: acetylcholine; CNS: central nervous system; E: epinephrine; Na+: sodium; NE: norepinephrine (adapted from Floras, 1993).

Creager *et al.*, 1990). The chronotropic response to changes in arterial and carotid sinus pressure induced by drug infusions (vasodilators) (Levine *et al.*, 1986), during upright tilt (Levine *et al.*, 1983), or by use of a neck chamber (Sopher *et al.*, 1990) specified to study carotid sinus baroreceptor reflex function, is attenuated in patients with heart failure. Also, cardiac sympathetic responses to acute vasodilation, assessed by measuring cardiac NA spillover, are reduced in patients with CHF compared with normal controls (Newton *et al.*, 1996). These data indicate that abnormal cardiopulmonary and arterial baroreceptor sensitivity may contribute importantly to the impaired sympatho-vagal balance seen in CHF and implicate at least one mechanism through which afferent modulation of sympathetic and parasympathetic efferent activity may be altered (*Creager et al.*, 1992). Attenuated baroreflex restraint underlies, to a great extent, sympathetic excitation in humans with CHF, given that sympathetic activity in skin, a tissue free of baroreflex restraint, was not increased in patients with heart failure (Middlekauff *et al.*, 1994). Baroreflex control of sympathetic nerve activity is impaired in early left ventricular dysfunction, even before generalised neurohumoral activation is present, indicating that baroreceptor dysfunction underlies sympathetic nervous system activation in CHF (Grassi *et al.*, 1995). There is experimental evidence that despite cardiopulmonary baroreflex attenuation in early left ventricular dysfunction, additional attenuation of arterial baroreflex control must be necessary for sympathetic excitation in heart failure (Kinugawa *et al.*, 1993). Abnormalities of reflex control of blood flow may be present in some organs but not others, as indicated by the intact cardiopulmonary baroreflex control of renal blood flow during non-hypotensive phlebotomy, despite the presence of blunted cardiopulmonary baroreflex control of forearm blood flow (Middlekauff *et al.*, 1995). Baroreceptor dysregulation of sympathetic nerve activity seems, therefore, to be organ specific, which may be related to differing thresholds in the nucleus tractus solitarius for baroreflex disinhibition of efferent sympathetic nerve activity for each organ (Middlekauff, 1997).

Diminished baroreflex sensitivity, representing reduced vagal reflexes, may be of prognostic significance not only in patients with asymptomatic left ventricular dysfunction (La Rovere *et al.*, 1988) but also in patients with symptomatic heart failure (Osterziel *et al.*, 1995). In moderate to severe CHF, a depressed sensitivity in vagal reflexes, which leads to a reduction in the tonic restraining influence on the sympathetic nervous system, parallels the deterioration of clinical and haemodynamic status and is significantly associated with poor survival (Mortara *et al.*, 1997a), suggesting that analysis of vagal reflexes may be a useful test to be added for stratification purposes in CHF. Particularly in patients with severe mitral regurgitation the baroreceptor modulation of heart rate (either as blunted reflex heart rate response or even a paradoxical tachycardia to blood pressure increase) appears as a powerful and independent predictor of cardiac mortality. In CHF, the mechanism of arterial baroreflex dysfunction is probably multifactorial and may be located in all components of the reflex arc (Porter *et al.*, 1990; Eckberg *et al.*, 1992). Thus, apart from the impaired ability of cardiovascular regulatory system to increase afferent vagal activity, patients with CHF seem also to have abnormalities of sinus node responsiveness to

changes in efferent traffic to the heart (White, 1981). Furthermore, increased plasma levels of angiotensin II (through activation of the RAA system in CHF) may act on baroreflex control of sympathetic tone and heart rate both directly in the vasomotor and cardiac centres in the brain and in the peripheral nerve terminals, facilitating NA and inhibiting acetylcholine release (Townend *et al.*, 1995). On the contrary, ACE inhibitors augment baroreflex control of sympathetic nerve activity in dogs with heart failure (Dibner-Dunlap *et al.*, 1996). Since, however, sympathetic excitation precedes plasma RAA system activation, it is difficult to implicate this system in the initiation of baroreflex dysfunction.

Some heart failure patients respond paradoxically to autonomic perturbations. They may have forearm vasodilatation and heart rate slowing rather than speeding during head-up tilt (Kassis, 1987; Nishian *et al.*, 1993); they may also vasodilate rather than vasoconstrict (Atherton *et al.*, 1997) and may not increase or even decrease their whole-body NA spillover (Davis *et al.*, 1987) or muscle sympathetic nerve activity (Ferguson *et al.*, 1992) as a response to reduction of central blood volume (i.e. during lower body suction). A hypothesis has been recently tested (Atherton *et al.*, 1997) that heart failure patients vasodilate during lower body suction because their ventricles interact during diastole such that decreases in right ventricular volume lead to reciprocal increases of left ventricular volume and, therefore, increased firing of left ventricular receptors reducing sympathetic nerve activity.

2. *Excitatory Afferents (Peripheral Chemoreceptors, Muscle Ergoreceptors, Cardiac Sympathetic Afferents)*

Even complete denervation of the baroreceptors, however, does not lead to such persistent sympatho-excitation as observed in CHF. Moreover, the gain of this reflex is known to be dramatically impaired in CHF (Smith *et al.*, 1989), thus making the arterial baroreflex loop unable to respond acutely by increasing sympathetic outflow. Also the underlying cause of the baroreceptor inhibition in the first place has not been clarified, although, according to a hypothesis (Packer, 1992b), prolonged atrial distension seems to lead to structural and functional changes in atrial receptor endings (Hirsch *et al.*, 1987). There is growing evidence that two other mechanisms may substantially contribute to the understanding of the persistent sympatho-excitation: the skeletal muscle ergoreflex (Piepoli *et al.*, 1996) and the arterial chemoreflex (Chua *et al.*, 1997). Skeletal myopathy and an interaction between arterial chemoreflex and cardiovascular autonomic centres, by exaggerating the sympathetic nervous responses to exercise, may contribute to the progression of the disease through an exaggeration of vasoconstriction in distant vascular beds (afterload enhancement) and through the direct myotoxic effects of prolonged sympatho-excitation.

a) Peripheral chemoreflex. A link between enhanced peripheral chemosensitivity and impaired autonomic control, expressed as either an abnormal profile of heart rate variability (HRV) or a depressed arterial baroreceptor sensitivity, has been recently demonstrated (Ponikowski *et al.*, 1997b). Peripheral chemoreflex may be important in the pathophysiology of CHF, given that patients with an augmented peripheral chemoreflex are more likely to exhibit abnormal ventilatory response to exercise (exercise hyperpnoea) and develop more often episodes of non-sustained ventricular tachycardia, possibly related to the chemoreflex-

driven sympathetic hyperactivity (Chua *et al.*, 1997). However, administration of oxygen to patients with heart failure is not associated with reduction in muscle sympathetic nerve activity, suggesting that tonic activation of the chemoreceptors is not the mechanism of chronic sympathoexcitation in CHF (Van de Borne *et al.*, 1996). Although the issue of whether augmented chemosensitivity is the cause or effect of increased sympathetic activity has not been clarified, the deleterious interactions between them may contribute to further autonomic imbalance in CHF. Augmented chemoreceptor drive, possibly due to the reduced blood flow to the carotid bodies, may represent a central factor affecting baroreflex sensitivity given that neurophysiological studies indicate that carotid baroreceptors and chemoreceptor neurons are distributed in close proximity to each other in the solitary and paramedian reticular nuclei in the medulla and that interneuronal connections might facilitate inhibitory interaction between these two reflexes (Miura *et al.*, 1972). Also, a low-frequency modulation of sympathetic nerve activity is present in patients with CHF and correlates with a low-frequency modulation of the respiratory cycle (reminiscent of Cheyne-Stokes breathing), indicating that altered chemoreceptor sensitivity and breathing may have important effects on sympathetic activation in CHF, if not causative, at least once it has already been established (Nguyen *et al.*, 1996).

b) Muscle ergoreflex. Unmyelinated and small myelinated afferents arise from poorly characterised work sensitive receptors within skeletal muscle, travel in the lateral spinothalamic tract and mediate an ergoreflex effect during exercise constituting an activation of the sympathetic vasoconstrictor drive (and possibly a small increase in heart rate) and an increase in respiratory frequency and ventilation in both normal people and patients with heart failure (Tibes, 1977; Piepoli *et al.*, 1995). These ergoreceptors, sensitive to the metabolic state of the muscle, are particularly active in patients with CHF and their contribution to the autonomic, haemodynamic and respiratory responses to exercise is enhanced in CHF compared with normal subjects (Piepoli *et al.*, 1996). Thus, skeletal muscle neural signals contribute directly to the perception of both cardinal symptoms muscle fatigue and dyspnoea and to excessive sympathetic outflow, producing vasoconstriction in distant non-exercising vascular beds, via an exaggerated ergoreflex activation in patients with CHF. A "muscle hypothesis" is, therefore, proposed as the basis of the generation of exercise intolerance and progression of the disease in CHF (Figure 1.7). In this hypothesis, which proposes another cycle of deterioration similar to that of neuroendocrine activation, left ventricular dysfunction leads to a catabolic state, muscle wasting and muscle metabolic abnormalities that are unmasked during exercise. Muscle myopathy in turn leads to exercise intolerance and sympathoexcitation associated with increased systemic vascular resistance, further contributing to the catabolic state (via reduced peripheral blood flow) and left ventricular dysfunction. Consequently, the combined effects of a persistent catabolic state and of a profound inactivity characterising heart failure further worsen skeletal muscle structure and function and may eventually lead to maladaptive left ventricular remodeling (Coats *et al.*, 1994). The time course of muscle ergoreflex abnormalities may be of pathophysiological significance before the onset of overt

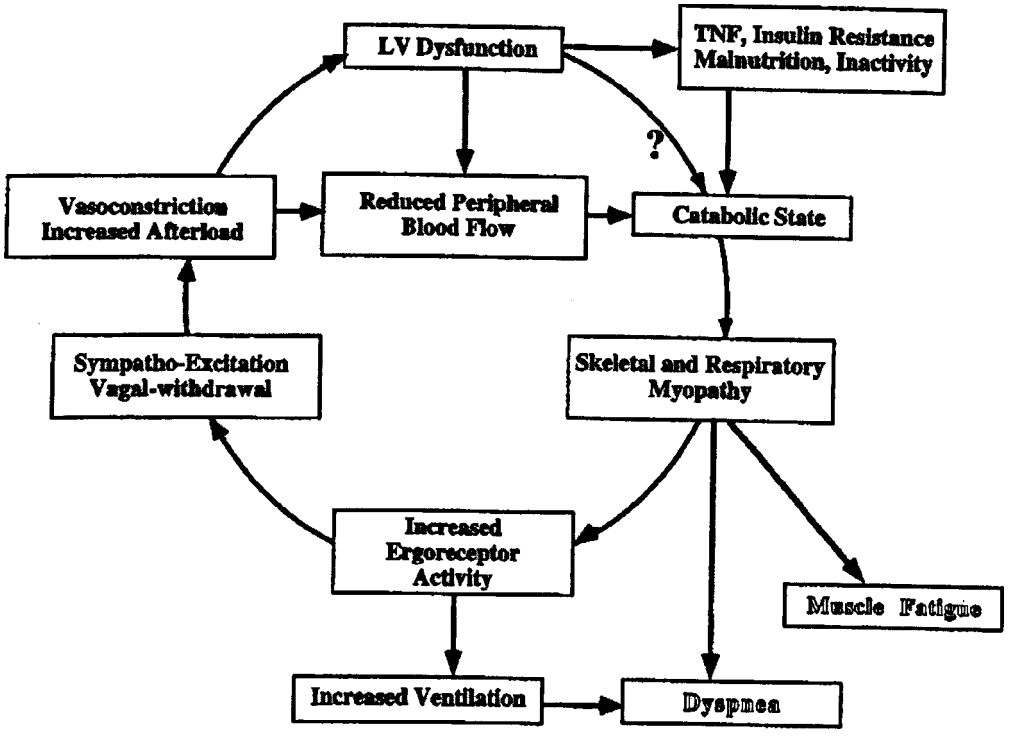

Figure 1.7. The "muscle hypothesis" as the basis of the generation of exercise intolerance and progression of the disease in CHF. In this hypothesis, which proposes another cycle of deterioration similar to that of neuroendocrine activation, left ventricular dysfunction activates catabolic and reduces anabolic factors and therefore leads to a catabolic state, muscle wasting and muscle metabolic abnormalities that are unmasked during exercise. Muscle (both skeletal and respiratory) myopathy in turn sensitises muscle "ergoreceptors", which leads to exercise intolerance and sympathoexcitation associated with increased systemic vascular resistance, further contributing to the catabolic state (via reduced peripheral blood flow) and left ventricular dysfunction. Consequently, the combined effects of a persistent catabolic state and of a profound inactivity characterising heart failure further worsen skeletal muscle structure and function and may eventually lead to maladaptive left ventricular remodeling (adapted from Coats *et al.*, 1994). TNF indicates tumor necrosis factor.

CHF and muscle wasting, since it has been shown that sympathetic activation is present early in asymptomatic left ventricular dysfunction.

c) Cardiac sympathetic afferents. Increased sympathetic efferent nerve activity may be originated from activation of cardiac sympathetic afferent nerves. Thus, efferent renal sympathetic nerve activity was enhanced in dogs with heart failure in response to epicardial bradykinin and capsaicin administration, whereas this effect could be attenuated by local lidocaine application (Wang *et al.*, 1996). Although the mechanisms underlying augmented sensitivity of sympathetic afferents are, to a great extent, unknown, intravenous indomethacin blocked the response to bradykinin, implicating a prostaglandin contribution.

3. *Abnormalities in Central Nervous System Control of Sympathetic Outflow*

Potential mechanisms of central abnormalities of neural regulation, resulting in enhanced central sympathetic neural outflow in heart failure, include: 1) central activation of angiotensin II, 2) blunted nitric oxide-mediated inhibition of central sympathetic neural

outflow, 3) abnormal insulin or opiate control of central sympathetic outflow, 4) activation of excitatory neurotransmitters, such as central monoaminergic systems. Thus, increased central nervous system turnover of catecholamines has been recently described and this was associated with an increase in cardiac NA spillover (Lambert *et al.*, 1995), and 5) release of endogenous brain ouabain-like activity producing sympathoexcitation. Experimental studies report (Leenen *et al.*, 1995) that intracerebroventricular administration of Digibind (digoxin immune Fab) caused a reduction in plasma catecholamine levels and eliminated the exaggerated sympathetic responses to air stress in rats with heart failure, thus implicating ouabain-like activity as a potential new mechanism to explain sympathetic excitation in CHF.

4. *Assesssment of Sympatho-vagal Balance*

Although the investigation of sympatho-vagal balance is limited by the lack of precise and quantifiable methods, autonomic function is currently assessed by a) plasma NA levels which are increased in patients with CHF but it is not clear whether this is due either to an enhancement in sympathetic activation or to alterations in neuronal uptake or clearance mechanisms for NA released from the nerve endings (Folkow *et al.*, 1983; Floras *et al.*, 1986), b) whole-body NA spillover estimating the rate by which NA released enters plasma, thus overcoming the confounding factor of impaired clearance in CHF but even this method is not a direct measure of sympathetic activity, as it cannot differentiate between different causes for the increase in the rate at which NA spills over from the nerve terminals (Esler *et al.*, 1988; Hasking *et al.*, 1986), c) heart rate variability using non-spectral (time domain) and power spectral (frequency domain) techniques (Floras *et al.*, 1988, Malliani *et al.*, 1991, Kienzle *et al.*, 1992). Power spectral analysis is a mathematical technique in which fluctuations of R-R intervals around the mean heart rate are analysed in the frequency domain and provide two major spectra (peaks): a higher frequency (HF) peak related to respiration and a low-frequency (LF) peak unrelated to any respiratory event which is further subdivided into a low- and very low-frequency components with some clinical and prognostic significance. Experimental work on parasympathetic and ß-blockade in dogs and humans indicates that the HF rhythm is entrained by the respiratory frequency and carried almost entirely by vagal activity, whereas the LF rhythm is mediated jointly by the vagal and sympathetic nervous system but predominantly by the latter (Pagani *et al.*, 1986; Malliani *et al.*, 1994). Mostly the ratio between HF and LF power is used to better describe the sympathetic activity. However, no gold standard of autonomic balance exists and the power spectral technique gives qualitative and semiquantitative information, which should be combined with other autonomic measures to describe autonomic nervous control of the cardiovascular system, d) assessment of sympathetic nerve traffic to muscle or skin, by using a microneurographic technique, permits direct quantitation of sympathetic nerve firing and its reflex control (Wallin *et al.*, 1988) (Figure 1.8). Recordings, however, are limited to superficial nerves innervating muscular or cutaneous vascular beds. Although muscle sympathetic nerve burst frequency does not necessarily represent firing rates in other

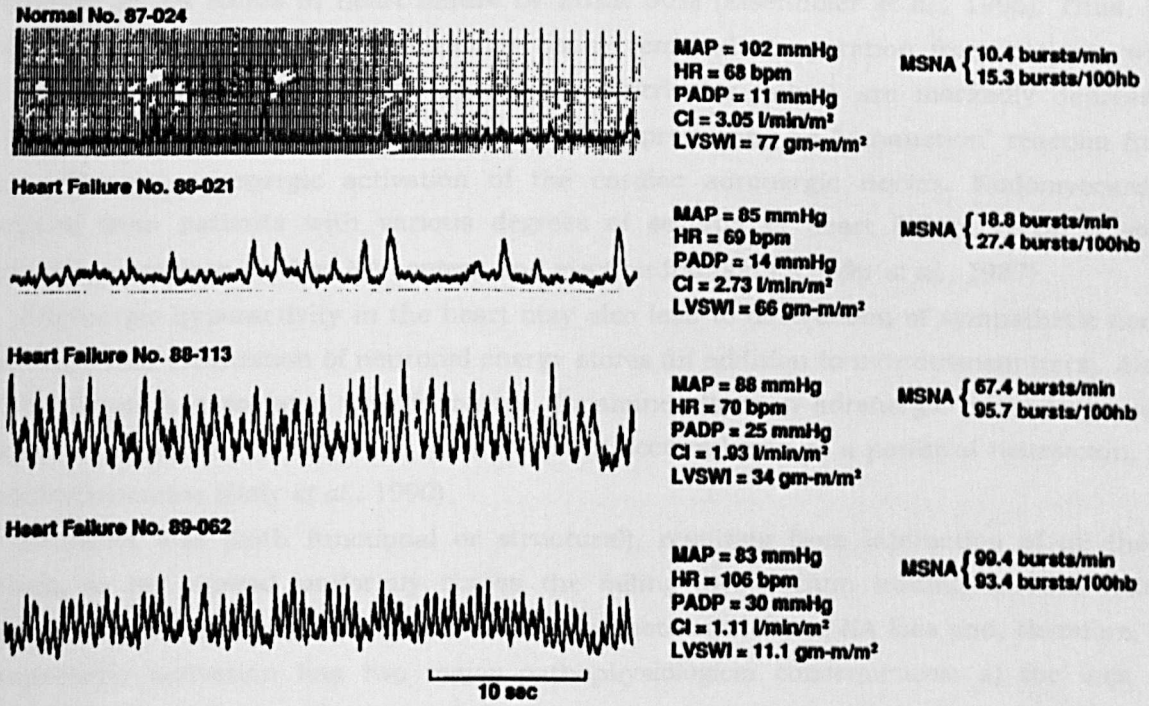

Figure 1.8. Microneurographic recordings of efferent muscle sympathetic nerve activity (MSNA). Sympathetic nerve traffic to muscle of a normal subject (upper panel), a patient with mild heart failure (second panel) and two patients with more advanced heart failure (lower two panels). The heart failure patients show significantly increased sympathetic burst frequency in the resting state compared with normal subject. Muscle sympathetic nerve firing rates are proportional to increasing severity of impairment of cardiac performance. bpm: beats per minute; CI: cardiac index; hb: heart beats; HR: heart rate; LVSWI: left ventricular stroke work index; MAP: mean arterial pressure; PADP: pulmonary artery diastolic pressure (adapted from Ferguson, 1993).

sympathetic nerves, there is evidence that a common mechanism influences the strength of sympathetic discharge to the heart and skeletal muscle (Wallin *et al.*, 1992).

Impaired sympatho-vagal balance in CHF is manifested by elevation of NA plasma levels and NA spillover as well as by reduced HRV. The extent of elevation of plasma NA concentration in patients with CHF correlates with the severity of left ventricular dysfunction.

5. *Myocardial Adrenergic Nervous Activity*

Myocardial adrenergic nervous system integrity is impaired during heart failure. This was initially attributed to a reduction in synthesis of NA through reduction in the activity of cardiac tyrosine hydroxylase (rate-limiting step in NA synthesis) (Pool *et al.*, 1967) or was thought to be secondary to a decrease in the number of normal nerve endings (Spann *et al.*, 1965). However, enhanced cardiac NA turnover is now known to be an important feature of CHF, in keeping with the observation of increased cardiac adrenergic drive preceding generalized sympathetic activation in human heart failure (Rundqvist *et al.*, 1997). A combination, therefore, of a reduced uptake and increased release of NA from adrenergic nerve endings has been reported in patients with congestive heart failure (Swedberg *et al.*, 1984). The chronically increased turnover with inadequate reuptake and storage results in a

reduction of NA stores in heart failure by about 50% (Eisenhofer *et al.*, 1996). Thus, NA concentrations in atrial and ventricular tissue removed at operation from patients with heart failure or from dogs with pure right ventricular failure are markedly depressed (Chidsey *et al.*, 1964, 1965 and 1966), possibly representing an "exhaustion" reaction from the prolonged adrenergic activation of the cardiac adrenergic nerves. Endomyocardial biopsies from patients with various degrees of severity of heart failure reveal a good correlation between cardiac NA content and ejection fraction (Schofer *et al.*, 1987).

Adrenergic hyperactivity in the heart may also lead to destruction of sympathetic nerve terminals and exhaustion of neuronal energy stores (in addition to neurotransmitters). Also, NA depletion is associated with increased dopamine stores in adrenergic nerve terminals, which is susceptible to oxidation with resultant accumulation of a powerful neurotoxin, 6-hydroxydopamine (Daly *et al.*, 1990).

Neuronal loss (both functional or structural), resulting from interaction of all these factors, is not spread uniformly across the failing myocardium leading to both richly innervated and markedly denervated areas. This heterogeneity of NA loss and, therefore, of sympathetic activation has two major pathophysiological consequences: a) the lack of temporal coordination of myocardial contraction and relaxation may contribute to deterioration in cardiac performance (Brutsaert, 1987) and b) the marked heterogeneity of resting potentials and action potential duration combined with the structural heterogeneity, that creates a conductive environment for re-entrant circuits, and with the increased triggered activity probably lead to the enhanced risk of sudden death characterising patients with CHF (Gelbland *et al.*, 1973; Wit *et al.*, 1977).

Although local myocardial NA stores do not seem to affect the intrinsic contractile state of the cardiac muscle (Spann *et al.*, 1966), cardiac NA depletion may contribute to the blunted response to stimulation of the cardiac sympathetic nervous system. Thus, the increments in contractile force and heart rate following stimulation of the cardiac sympathetic nerves in experimental heart failure with cardiac NA depletion are either abolished or much smaller compared with normal animals (Covell *et al.*, 1966). Chronotropic response to infusion of isoproterenole and exercise as well as contractile sensitivity may be further attenuated by the postsynaptic desensitisation of the ß-adrenergic receptor pathway, due to the combined effect of ß-adrenergic receptor down-regulation and increase in the inhibitory G protein observed in patients with congestive heart failure (Colucci *et al.*, 1989). More recently, iodine-123 metaiodobenzylguanidine (MIBG) imaging reflected accelerated myocardial adrenergic nerve activity in proportion to the severity of heart failure, independent of the underlying cause (Imamura *et al.*, 1995). Analysis of iodine-123 MIBG, an analog of the adrenergic blocking agent guanethidine which shares many cellular transport properties with NA (Wieland *et al.*, 1980), represents a useful, non-invasive tool for assessing the disturbances in adrenergic function and evaluating the severity of heart failure.

ii. The Renin-Angiotensin-Aldosterone (RAA) System

Early in the course of left ventricular dysfunction, in animal models of heart failure, the RAA system is suppressed, probably by ANP (Luchner *et al.*, 1996). Enhanced tone of sympathetic nervous system and increased release of ANP and AVP may precede activation of the RAA system, which is dramatically augmented by the first use of diuretics. Lack of substantial activation of the RAA system in mild-untreated heart failure may be partly due to suppresion of initial activation of this system by expansion of the circulating volume and sodium retention (Poole-Wilson, 1996). When cardiac output falls, systemic perfusion pressure is maintained by peripheral vasoconstriction and sodium retention. Both mechanisms are, to a great extent, the result of direct and indirect effects of RAA system on peripheral vascular beds and on glomerular and tubular function. At that stage RAA axis operates in concert with the activated adrenergic nervous-adrenal medullary system to preserve systemic perfusion pressure and a reasonable relationship between further increases in circulating renin and angiotensin II levels and the severity of heart failure has been observed.

Stimulation of β_1-adrenoceptors in the juxtaglomerular apparatus by an enhanced sympathetic drive, activation of the baroreceptors in the renal vascular bed by a reduction of renal blood flow and decreased delivery of sodium to the macula densa (following salt restriction and diuretic treatment) are the major factors contributing to the release of renin in CHF. In addition, all the circulating components of the RAA system also exist in tissue sites and there is growing evidence of activation of these local tissue systems in the heart, kidney, brain and blood vessel walls. Stepwise activation of RAA system exhibits organ specificity. Thus, in experimental heart failure increased levels of mRNA for ACE are observed in the heart but not in the kidneys or the lungs (Pieruzzi *et al.*, 1995), whereas in overt CHF both cardiac and renal angiotensin II levels are increased (Luchner *et al.*, 1996). Although the role of these local systems in the clinical expression and the progression of the syndrome remains unknown, some of the beneficial effects of the ACE inhibition may be due to the interruption of the RAA local tissue systems.

At an organ level, elevated local and circulating angiotensin II can act on a) the adrenal cortex to release aldosterone with the potent sodium retaining properties, b) the posterior pituitary to release AVP, c) resistance arterioles to produce peripheral vasoconstriction, d) the renal mesangial tissue to reduce filtration coefficient and e) the efferent glomerular arteriole to increase intraglomerular pressure and filtration (Francis, 1989). Angiotensin II also enhances the adrenergic nervous system's biosysthesis and release of NA and is a potent dipsogenic amine by stimulating the cerebral thirst centre. In the myocardium, RAA system causes myocyte hypertrophy, necrosis and apoptosis (Tan *et al.*, 1991; Eichhorn *et al.*, 1996).

It appears that tissue RAA system and local angiotensin II production may also have important functional roles and plasma ACE activity may not reflect tissue ACE activity (Swedberg *et al.*, 1990). Therefore, vascular renin-angiotensin activity and local production of angiotensin II may contribute to the maintenance of arterial tone. In patients with CHF

administration of an ACE inhibitor produced vasodilatation at doses that did not have any systemic effects and improved venodilatation associated with reduction in intravascular volume (Raya *et al.*, 1991; Qing *et al.*, 1992). The contribution of NO and bradykinin in coronary circulation to ACE inhibitor-induced vasorelaxation indicates that mechanisms other than blockade of angiotensin II may also play a role in its vasoprotective effects (Sudhir *et al.*, 1996). It is possible that some of the beneficial effects of ACE inhibitors in patients with clinically overt heart failure (The AIRE Study, 1993) or laboratory assessed left ventricular dysfunction (Pfeffer *et al.*, 1992) may be mediated via an increase in myocardial blood flow.

Although less is known about the involvement of the cardiac RAA system in CHF, angiotensin II taken up from the circulation or generated in the heart may mediate the cardiac hypertrophic response to increased cardiac load (Ruzicka and Leenen, 1995). Angiotensin II has been found to promote growth of cardiac myocytes (Naftilan *et al.*, 1989), which may be facilitated by the angiotensin II-induced augmented NA release from sympathetic nerve endings. There is also evidence that the RAA system is responsible for the stimulation of fibrosis, especially in advanced CHF; angiotensin II and aldosterone stimulate collagen synthesis in cultured fibroblasts and angiotensin II inhibits collagen degradation (Brilla *et al.*, 1993 and 1994). In cardiac myocytes and fibroblasts (cultured cells) obtained from the neonatal rat, angiotensin II caused myocyte hypertrophy and fibroblast proliferation associated with the induction of mRNA for several early response genes (*c-fos, c-jun, Jun B, Egr-1, c-myc*), angiotensinogen and the peptide growth factor transforming growth factor beta1, indicating that angiotensin II can exert significant myocardial effects independent of changes in loading conditions on the heart (Sadoshima *et al.*, 1993; Crawford *et al.*, 1994). Thus, enhanced local synthesis of angiotensin II in the myocardium may be involved in the process of ventricular remodeling that occurs with cardiac dilation in patients with CHF via cell growth, collagen deposition and apoptosis. In keeping with this hypothesis, some investigators (Sharpe *et al.*, 1991; Greenberg *et al.*, 1995) demonstrated that treatment of patients with left ventricular dysfunction with an ACE inhibitor decreases the end-systolic and end-diastolic volumes compared with placebo groups. However, differences in the affinity for cardiac tissue ACE may determine the effect of various ACE inhibitors on prevention of hypertrophy and remodeling of the heart in response to pressure or volume overload (Ruzicka and Leenen, 1995).

The role of the cardiac RAA system in the genesis and increased incidence of ventricular tachyarrhythmias may be important in patients with CHF. Infusion of angiotensin I or II increased the occurence of ventricular arrhythmias under ischaemic conditions in an animal model and was abolished by an ACE inhibitor (Linz *et al.*, 1986). This observation was further supported in a large scale trial (Cohn for the V-HeFT II study, 1991), which showed that patients receiving an ACE inhibitor experienced a greater reduction in mortality due to arrhythmic deaths when compared with patients receiving a pure vasodilating combination. These results were not confirmed by the SOLVD investigators (1991), who

failed to demonstrate a similar reduction in mortality due to a decrease in sudden cardiac death; the study design, however, differed between those two mega-trials.

It is not surprising, therefore, that interruption of the RAA axis with ACE inhibitors elicits a mild diuretic effect (by lowering angiotensin II-stimulated production of aldosterone) but mainly reduces systemic vascular resistance as well as afterload and thereby increases cardiac output in CHF. In addition, regression of fibrosis has been demonstrated with ACE inhibitor treatment (Baig *et al.*, 1998). It must be emphasised, however, that the way they mediate their beneficial effects is not clear, whether by reduced circulating or tissue-based angiotensin II or by enhancement of bradykinin or other kinin systems. Recent experimental evidence with angiotensin II type 1 receptor blockade indicates that the interaction of angiotensin II and bradykinin appears to be essential for the development and maintenance of a normal heart (Madeddu *et al.*, 2000).

iii. Arginine-Vasopressin (AVP) System

Circulating AVP is found in elevated plasma concentrations (twice normal levels) in many patients with CHF, even after correction for plasma osmolality (Goldsmith *et al.*, 1983 and 1986). Although some investigators believe that an increase in AVP levels is in direct proportion to haemodynamic and clinical severity of heart failure (Yamane *et al.*, 1968), recent investigations from the SOLVD registry failed to demonstrate a strong relation between AVP and ejection fraction (Benedict *et al.*, 1993). The precise mechanism for the release of this neurohormone from the posterior pituitary in patients with CHF is not well understood but probably involves non-osmotic factors based on the observation that patients with CHF fail to show the normal reduction in AVP levels with the reduction of their serum osmolality (Pruszczynski *et al.*, 1984). These factors include decreased sensitivity of atrial strech receptors and, therefore, impairment in baroreceptor-mediated inhibition of AVP release with atrial distension through brain stem centres (Zehr *et al.*, 1971; Greenberg *et al.*, 1973) as well as the increased angiotensin II levels, which may also directly stimulate the hypophysical production of the neurohormone (Francis, 1989). Plasma AVP levels are frequently increased in parallel to an increase in plasma renin activity and catecholamine concentrations due to compromised end-organ perfusion (Goldsmith *et al.*, 1983).

Although AVP levels are increased in patients with asymptomatic left ventricular dysfunction (Francis *et al.*, 1990a), with further increase when symptoms of heart failure develop, its importance in the pathophysiology of CHF is not certain. Neurohormone's actions are a combination of haemodynamic, with a profound arteriolar vasoconstriction, sometimes accompanied by a negative inotropic action (Liard, 1986; Francis, 1990b), and renal, with an action on the collecting duct to increase free water reabsorption and thereby antidiuresis, perhaps contributing to the hyponatraemia sometimes observed in advanced CHF. Administration of AVP antagonists reduces systemic vascular resistance and increases cardiac output in patients with CHF and elevated AVP. Two types of AVP receptors have been identified in several tissues: V_1 receptor contributing to systemic vasoconstriction and V_2 receptor regulating free water clearance (Braunwald, 1996).

iv. Natriuretic Peptides

C-terminal and N-terminal atrial natriuretic peptides (CT-ANP and NT-ANP) are synthesised in the granulated cells of atria and ventricles and released mainly in response to increased atrial stretching. Atrial stretch leads to a secretion of ANP, which inhibits the release of NA and its vasoconstricting actions on peripheral blood vessels and exerts direct vasodilator and natriuretic effects, thus reducing the haemodynamic load on the heart. Several investigators found ANP levels in plasma to correlate inversely with the level of ejection fraction (Hara *et al.*, 1987; Benedict *et al.*, 1993) or cardiac index (Rouleau *et al.*, 1988) and directly with the severity of CHF (Hara *et al.*, 1987). Furthermore, ANP plasma levels and more recently the N-terminal of the ANP free-hormone, characterised by longer half-life and greater stability compared with ANP, have been shown to be independent predictors of cardiovascular mortality in patients with left ventricular dysfunction and/or overt CHF (Gottlieb *et al.*, 1989; Hall *et al.*, 1994).

The release of ANP during asymptomatic left ventricular dysfunction, following atrial stretching due to an early increase in filling pressures, seems to influence regional blood flow and sodium retention, thus offloading the heart and, therefore, acting as a stress-lowering compensatory mechanism after a substantial myocardial injury. Prolonged atrial distension, however, leads to depletion of ANP, such that its release as a response to increase in atrial pressure is blunted and ANP is now synthesised by the ventricles as well as the atria in inadequate quantities (Packer, 1992b). Atrial natriuretic peptide is normally expressed in fetal but not adult ventricular myocardium. In CHF, however, both in patients and in experimental heart failure, ANP is synthesised in the ventricles as well as in the atria and in this rather advanced state the ventricles become an important source of circulating ANP (Franch *et al.*, 1988; Takemura *et al.*, 1989). Moreover, patients with overt CHF, despite the increase in ANP levels, continue to exhibit augmented renal, splachnic and forearm vascular resistance. Similarly, studies in patients with CHF show little change in renin release or sodium excretion following infusion of exogenous ANP or administration of neutral endopeptidase (producing peptide's breakdown) inhibitors resulting in enhanced endogenous ANP levels. This indicates that not only is the release of ANP blunted after long-term atrial distension but also the peptide, once released, loses its ability to supress the RAA system and to produce peripheral vasodilation, finding compatible with a down-regulation of end-organ sensitivity to the action of this counter-regulatory hormone (Cody *et al.*, 1986; Kohzuki *et al.*, 1989). There is evidence, however, that administration of an ANP receptor antagonist (Wada *et al.*, 1994) or endopeptidase inhibitor (Münzel *et al.*, 1992), despite attenuated haemodynamic and renal effects, the peptide continues to exert a suppressive effect on the renin activity and a reduction in NA and AVP plasma levels associated with a reduction in right and left heart filling pressures. A potential sympathomodulatory role for endogenous ANP in mild to moderate CHF and a potential therapeutic role for exogenous ANP emerges from a recent study which is consistent with the concept that ANP exerts a sympathoinhibitory action in heart failure (by measuring muscle sympathetic nerve activity), especially in response to reductions in atrial pressures

that do not affect systemic blood pressure (Abramson *et al.*, 1999). There is, also, evidence that natriuretic peptides may directly inhibit vascular smooth muscle cell and/or myocardial cell hypertrophy as well as DNA synthesis in cardiac fibroblasts (Itoh *et al.*, 1990; Calderone *et al.*, 1994; Cao and Gardner, 1995).

There is increasing interest in the measurement of brain natriuretic peptide (BNP), presenting a high level of homology with ANP, as a marker for early ventricular enlargement in asymptomatic patients with left ventricular dysfunction or patients with mild CHF. Measurement of BNP has been reported (McDonagh *et al.*, 1998) to be a cost-effective method of screening for left ventricular dysfunction in the general population, especially if its use is restricted to individuals at high risk (ie. older people with ischaemic heart disease). A raised BNP concentration seems to be more accurate than a raised NT-ANP in detection of left ventricular systolic dysfunction (McDonagh *et al.*, 1998). Brain natriuretic peptide, stored mainly in the ventricular myocardium and released as a response to alterations in ventricular filling pressures, causes, also, natriuresis and vasodilatation (Moe *et al.*, 1993). Despite a low level of BNP expression in normal human hearts, atrial content of BNP increase 10-fold and circulating levels of BNP are elevated in patients with CHF (Wei *et al.*, 1993). Both plasma cardiac natriuretic peptides, ANP (mainly from the atrium) and BNP (mainly from the ventricle), are increased with the severity of CHF due to left ventricular dysfunction and these increases are correlated to the hemodynamic parameters such as pulmonary capillary wedge pressure (PCWP) and left ventricular ejection fraction (LVEF) (Yoshimura *et al.*, 1993). The compensatory activity of the cardiac natriuretic peptide system is attenuated as the syndrome progresses and mortality increases in CHF patients with high plasma levels of ANP and BNP (Tsutamoto *et al.*, 1997). It has been recently reported that the 76-amino acid residue amino terminal portion of pro-BNP (N-BNP) circulates in human plasma and that the levels are elevated in cardiac impairment (Hunt *et al.*, 1997).

There is growing evidence that among plasma cardiac peptides, cGMP, adrenomedullin (ADM) and catecholamine levels, the ventricular hormones N-BNP and BNP best reflect left ventricular function and provide prognostic information (independent of age, sex, clinical history and LVEF) regarding the risk of death in patients with CHF (Tsutamoto *et al.*, 1997) or the risk of death or heart failure in the 2 years following myocardial infarction (Richards *et al.*, 1998). Stratification of patients into low- and high-risk groups can be facilitated by plasma N-BNP or BNP measurements and one of these variables could be included in the routine clinical workup of patients after myocardial infarction. Why are plasma BNP or N-BNP stronger prognostic predictors and more sensitive markers of ventricular damage than ANP or LVEF? The most likely explanation is that ventricular natriuretic peptides play a role as an emergency aid for ANP in patients with left ventricular dysfunction and that synthesis and secretion of BNP or N-BNP are stimulated with the degree of myocardial ischaemia, necrosis, damage and local mechanical stress on ventricular myocytes even when the global haemodynamic condition remains unchanged (Hama *et al.*, 1995; Tsutamoto *et al.*, 1997).

F. Effects of Excessive Neurohormonal Activation in Heart Failure

Neurohormonal activation in the early stages of CHF plays a compensatory role, supporting the failing heart by increasing heart rate and enhancing myocardial contractility. Thus, arteriolar vasoconstriction maintains blood pressure and vital organ perfusion, whereas venoconstriction augments venous return and cardiac filling pressures and invokes Frank-Starling principle. In addition, adrenergic stimulation enhances diastolic ventricular function via ß-receptor-mediated increases in cyclic adenosine monophosphate, with subsequent phosphorylation of troponin I, phospholamban and the calcium pump, thus leading to reduced myofilament calcium sensitivity, accelerated sequestration of calcium into the sarcoplasmic reticulum and increased removal of sarcoplasmic calcium (Katz, 1990; Walsh, 1990). These compensatory effects are eventually outweighed by a number of adverse consequences that serve to exacerbate the CHF syndrome. Generalised neurohormonal excitation at rest and exacerbation of the autonomic nervous system responses to exercise may contribute to the progression of the syndrome's process through an exaggeration of peripheral vasoconstriction and through direct myotoxic effects of prolonged sympathoexcitation.

i. Peripheral Vasoconstriction

Nearly all neurohormonal systems that are activated in CHF exert potent vasoconstrictor effects on peripheral blood vessels which are not counterbalanced by endogenous vasodilators. Thus, circulating, such as NA, angiotensin II and vasopressin, as well as locally active vasoconstrictors, such as endothelin-1 (enhanced by catecholamines and angiotensin II), are increased (Margulies *et al.*, 1990; Packer, 1992b; Joseph *et al.*, 1998), whereas the actions of circulating, such as ANP and prostaglandins, as well as locally active vasodilators, such as EDRF, are attenuated (Cody *et al.*, 1986; Kubo *et al.*, 1991; Packer, 1992b). Recent studies suggest that increased circulating endogenous insulin levels (Houghton *et al.*, 1998) and endothelial release of prostaglandins (Lang *et al.*, 1997) contribute to skeletal muscle arteriolar vasodilation in patients with CHF. In addition to neurohormonal activation, increased peripheral vascular resistance, characterising CHF, may be due in part to impaired EDRF activity (Katz *et al.*, 1992). Endothelium-dependent vasodilation and endothelial control of conduit artery distensibility have both been reported to be abnormal in CHF (Lindsay *et al.*, 1992b; Ramsey *et al.*, 1995). Potential mechanisms for endothelial dysfunction in CHF include increased levels of cytokines (eg, TNF-a) impairing the synthesis of NO synthase and causing endothelial-cell apoptosis, increased ACE activity facilitating breakdown of bradykinin, increased levels of oxygen radicals inactivating EDRF, chronically reduced blood flow decreasing expression of endothelial NO synthase and impaired endothelial receptor signal transduction pathways (Drexler, 1995; Ferrari, 1998a).

Peripheral vascular resistance is also increased by mechanical factors such as sodium retention, due to sustained neurohormonal activation, and changes in the structural

components of the vessel walls, possibly due to long-term decreases in regional blood flow (Creager *et al.*, 1991). Salt and water retention may impair the vasodilator capacity of peripheral blood vessels either because of an increase in the sodium content of the peripheral vessel walls or because of an oedema-induced enhancement of the compressive forces of perivascular tissues (Sinoway *et al.*, 1987).

Reduced total peak blood flow to the exercising limb may activate skeletal muscle ergoreflex, in response to early local metabolic distress, leading to excessive sympathetic vasoconstrictor drive to non-exercising muscle beds and possibly contributing to dyspnoea and fatigue, the cardinal symptoms in CHF (Zelis and Flaim, 1982; Coats *et al.*, 1994; Piepoli *et al.*, 1995).

The development of peripheral vasoconstriction and sodium retention in the course of CHF both herald the initiation of a state of decompensation with the endogenous mechanisms directed to preserve systemic perfusion pressures instead of supporting cardiac function.

ii. Myotoxic Effects

Excessive and prolonged sympatho-excitation may eventually lead to a progressive effect on remodeling of the left ventricle through an exaggeration of peripheral vasoconstriction and hence afterload and diastolic wall stress that develop. In addition, persistent sympatho-excitation and prolonged activation of the renin-angiotensin system may exert adverse effects on the myocardium itself independent of their haemodynamic actions.

I. First, alterations in the cardiac ß-adrenergic pathway, incuding down-regulation of ß-adrenergic receptors (mainly $ß_1$) and uncoupling of ß-receptors from their effector enzyme (adenylate cyclase), lead the failing heart to lose its responsiveness to the positive inotropic effects of endogenous and exogenous ß-receptor agonists (Colucci *et al.*, 1989; Bristow *et al.*, 1990). The $ß_1$ component of the inotropic response to adrenergic stimulation is markedly decreased and is associated with a compensatory increase in the $ß_2$ component ($ß_1$:$ß_2$ ratio decreased from 77:23 in the non-failing to 60:38 in the failing ventricle) (Bristow *et al.*, 1986; Joseph *et al.*, 1998). The molecular basis of agonist-induced ß-adrenergic receptor down-regulation may be related to RNA binding proteins that selectively bind to $ß_1$- and $ß_2$-receptor messenger RNAs sparing α_1-adrenergic receptor mRNA (Huang *et al.*, 1993). In patients with CHF, expression of the mRNA-binding protein AUF1, which decreases the stability of mRNA, is upregulated and associated with a significant reduction in mRNA for the $ß_1$-adrenergic receptor and subsequent down-regulation of the receptor (Pende *et al.*, 1996). Both a decrease in the guanine nucleotide binding protein that stimulates (G_s) and an increase in the binding protein that inhibits (G_i) the interaction of the ß-adrenergic receptor and its effector enzyme adenylate cyclase as well as a decrease in adenylate cyclase activity, have been reported in patients with CHF (Horn *et al.*, 1988; Neumann *et al.*, 1988; Bristow *et al.*, 1992). Although the mechanism responsible for the increase in the ratio G_i/G_s remains unknown, alterations in G protein function seem to be related to the cause

of heart failure. Idiopathic dilated cardiomyopathy is mainly associated with an increase in G_i activity and reduction in G_s function (Böhm *et al.*, 1990). Progressive decrease in ß-receptor density, assessed by endomyocardial biopsies, has been associated with increasing severity of heart failure (Fowler *et al.*, 1986). In parallel, endomyocardial biopsy ß-receptor density correlates with maximal oxygen consumption in patients with CHF, suggesting an important role of ß-receptor down-regulation in the impaired chronotropic and inotropic responses to peak exercise characterising this syndrome (White *et al.*, 1995). Drugs producing favourable effects in CHF, such as ACE inhibitors (lisinopril) and selective $ß_1$-blockers (metoprolol), alter cardiac adrenergic function and enhance ß-adrenergic receptor density (Gilbert *et al.*, 1993 and 1996). New generation of non-selective ß-blockers (carvedilol), however, lower cardiac adrenergic drive causing no change in ß-receptor expression in the heart and produce relatively greater improvements in indices of left ventricular function associated with improved survival (Gilbert *et al.*, 1996; Packer *et al.*, 1996).

II. Second, high concentrations of NA and angiotensin II exert direct toxic effects on myocardial cells causing altered sarcolemmal permeability and myocytolysis with subsequent replacement fibrosis (Tan *et al.*, 1991; Mann *et al.*, 1992). The magnitude of cell death is concentration dependent as shown in isolated cell culture (Mann *et al.*, 1992). Although the mechanism by which neurohormonal exposure leads to cell toxicity remains uncertain possibilities include a state of increased calcium influx and overload (as a result of ß-adrenoceptor-mediated cAMP-dependent activation of the slow calcium channel) and an enhancement of free radical production in the failing heart (Rona, 1985; Belch *et al.*, 1991). Calcium overload may lead to myocardial cell loss through ATP depletion (due to mitochondrial overload), release of intracellular phospholipases (impairing the integrity of the phospholipid cell membranes) and activation of endonucleases (responsible for mediating apoptotic cell death) (Mann, 1998). In addition, there is growing evidence that proinflammatory cytokines (eg, TNF-a), associated with increased neurohormonal activation (Levine *et al.*, 1990), inhibit myocardial contractility and this direct negative inotropic effect is largely mediated through myocardial inducible form of NO synthase (Finkel *et al.*, 1992). Induction of NO synthase activity in the heart, associated with a parallel increase in myocyte death (Pinsky *et al.*, 1995), has been reported in myocardial samples obtained from patients with dilated cardiomyopathy (de Belder *et al.*, 1993), suggesting that overproduction of NO, a gaseous free radical, may be a common pathophysiological mechanism leading to heart failure. Recent data, however, suggest that adrenergic activation is associated with marked increases in myocardial gene expression of TNF-a, interleukin (IL)-1ß and IL-6 independent of myocardial iNOS (Prabhu *et al.*, 2000).

III. Third, excessive and prolonged activation of sympathetic nervous and renin-angiotensin systems may provoke exacerbation of ventricular arrhythmias predisposing to sudden death by exerting adverse electrophysiological effects (possibly by altering intracellular cyclic AMP and potassium) (Packer, 1992b).

IV. Finally, enhanced and persistent adrenergic stimulation, through the direct effects of NA on a_1- and ß-adrenergic receptors located on cardiac myocytes, vascular smooth muscle cells, endothelial cells and fibroblasts, may induce the expression of a variety of peptide growth factors, thus affecting the growth and phenotype of the failing myocardium (Schneider and Parker, 1990; Long *et al.*, 1993). More specifically, in cardiac myocytes this expression is associated with the reappearance of fetal genes and their altered products such as fetal isoforms of proteins involved in the regulation of myocardial energetics and, therefore, the development of contractile force.

G. Neurohumoral Activation and Heart Failure: Summary

Evidence, therefore, from the recent literature supports the following hypotheses regarding the mechanisms and implications of autonomic nervous system dysfunction in heart failure:

I. Neurohumoral activation contributing to transition from asymptomatic left ventricular dysfunction to heart failure is stepwise and organ specific.

II. Abnormalities of the arterial and cardiopulmonary baroreceptors underlie and may predispose to the early sustained activation of the sympathetic nervous system in patients with asymptomatic left ventricular dysfunction.

III. The development of atrial natriuretic peptide resistance leads to marked activation of the sympathetic and the renin-angiotensin systems and transition to overt heart failure and abnormalities of the sympathetic control of the cardiovascular system.

IV. Augmented discharges from additional afferent systems more characteristic of rather advanced heart failure, such as the skeletal muscle ergoreceptors, peripheral chemoreceptors and cardiac sympathetic afferents, as well as the central nervous system, may be associated with further neurohumoral activation.

V. Therapeutic strategies interrupting or even reversing the neurohumoral activation in heart failure hold the greatest promise to improve quality of life and prolong survival in the growing patient population with this syndrome.

H. Physical Training and Autonomic Function in Heart Failure

Regular physical training has been shown to improve exercise tolerance in normal subjects and in patients with ischaemic heart disease (Clausen *et al.*, 1976), prevent the changes in body composition and fuel metabolism associated with ageing (Horber *et al.*, 1996), improve blood lipid profile, reduce susceptibility to ventricular arrhythmias and modify psychological status (Scheuer *et al.*, 1977; Clausen *et al.*, 1977; Blomqvist, 1983), all of which would be of considerable clinical and prognostic benefit in patients with CHF. Moreover, physical training results in diminished sympathetic responses for a given level of exercise, as indicated by the substantial reduction in blood catecholamine levels (by as much as 90% at heavy work rates), heart rate and systolic blood pressure in normal individuals (Cousineau

et al., 1977; Winder *et al.*, 1979; Casaburi *et al.*, 1987). This hormonal component of the training adaptation occurs very early in the course of a vigorous endurance training programme (Winder *et al.*, 1979). The observation of a strong link between sympathetic response and exercise requirements implies that sympathetic tone during work is subjected to precise control mechanisms (Péronnet *et al.*, 1981). Although the forwarded hypothesis that these control mechanisms originate in cardiovascular or muscular chemoreceptors or from the motor cortex deserves further investigation, there is growing evidence that muscle ergoreflex contributes significantly to the haemodynamic, autonomic and ventilatory responses to exercise in men (Piepoli *et al.*, 1995). Finally, endurance training enhances baroreflex sensitivity and HRV in normal subjects (Goldsmith *et al.*, 1992), borderline hypertensives (Somers *et al.*, 1991) and established hypertensives (Pagani *et al.*, 1988).

The benefits of localised conditioning (Minotti *et al.*, 1990b) or systemic exercise training (Sullivan *et al.*, 1988a) on skeletal muscle morphologic, histochemical and biochemical characteristics as well as functional and blood flow abnormalities in patients with CHF have been well documented. Although a subgroup of these patients showed improved central haemodynamics after training, the increases in systemic exercise performance have been primarily attributed to improvements in the peripheral mechanisms. Little, however, is known regarding the effects of exercise training programmes on autonomic control of the cardiovacsular system in heart failure. On the other hand, patients with CHF are characterised by a marked activation of various neurohormonal mechanisms, including enhancement of renin activity and elevation of circulating catecholamines, ANPs and AVP (Francis *et al.*, 1984). These autonomic changes increase peripheral vascular resistance and reduce blood flow to various organs (especially during exercise), including skeletal muscles. Moreover, the degree of elevation of NA and ANPs (Cohn *et al.*, 1984; Keogh *et al.*, 1990) or decrease in vagal tone, HRV and baroreflex sensitivity (Kleiger *et al.*, 1987; Saul *et al.*, 1988; Osterziel *et al.*, 1995; Ponikowski *et al.*, 1997a) has been shown to be independent risk factors for death. Physical training has been demonstrated to decrease rest plasma catecholamine levels in patients with ischaemic heart disease (Cooksey *et al.*, 1978), enhance HRV and improve baroreceptor sensitivity in hypertensives, reflecting an increase in vagal tone (Pagani *et al.*, 1988). Exercise conditioning increased the ventricular fibrillation threshold through a shift in sympatho-vagal balance towards vagal predominance in dogs with experimentally induced myocardial infarction (Billman *et al.*, 1984). An insight into the mechanisms underlying the beneficial effects of training is gained in an experimental model of acute myocardial ischaemia where 6 weeks of physical training produced a concomitant increase in baroreflex sensitivity, HRV and repetitive extrasystole threshold and protected all animals from recurrence of ventricular fibrillation during treadmill (Hull *et al.*, 1994). A training-induced reduction in adrenergic tone and increase in vagal tone has been associated with higher ventricular fibrillation threshold in trained dogs with a healed myocardial infarction (Vanoli *et al.*, 1991).

Recent meta-analysis of randomised trials of cardiac rehabilitation with exercise (Oldridge *et al.*, 1988; O'Connor *et al.*, 1989) indicate a moderate reduction in total and

cardiovascular mortalities persisting throughout the follow-up of patients after myocardial infarction. The correct identification of patients who might benefit most from an exercise training programme is still a subject of controversy. Exercise training may, however, prolong survival in post-myocardial infarction patients with depressed left ventricular function (Specchia *et al.*, 1996). Although this study does not elucidate the mechanism whereby training may prolong survival, a beneficial effect by inducing a change in the autonomic balance of the heart is strongly suggested. This speculation is based on the a) strong evidence linking the autonomic nervous system to cardiovascular mortality after myocardial infarction (La Rovere *et al.*, 1988), b) impairment of autonomic innervation to and from the heart induced by myocardial ischaemia and infarction, thus modulating the development of malignant arrhythmias and predisposing the occurence of sudden death (Webb *et al.*, 1972; Zipes, 1995), c) deterioration of cardiac function by worsening the process of ventricular remodeling as a result of elevated sympathetic activity causing an increase in wall stress and loading condition (Pilati *et al.*, 1992) and d) modification of sympatho-vagal balance toward a condition of parasympathetic dominance in normal subjects after physical training (Somers *et al.*, 1986b; Goldsmith *et al.*, 1992). This autonomic hypothesis is further supported by a recent study (Malfatto *et al.*, 1996) where exercise training, performed 8 weeks after a myocardial infarction, modifies the sympatho-vagal control of HRV (both in the time and frequency domain) toward a persistent enhancement in parasympathetic tone, known to be associated with a better prognosis. Besides preventing life-threatening arrhythmias in high risk patients, training-induced increase in markers of vagal activity (Hull *et al.*, 1994) may limit the deleterious effects of sympathetic hyperactivity on left ventricular performance, particularly in patients with low ejection fractions. A recent study demonstrates an improvement in the harmful process of left ventricular remodeling in postinfarction patients with left ventricular dysfunction by participation in a structured exercise rehabilitation programme, possibly due to a chronic reduction in sympathetic tone (Giannuzzi *et al.*, 1997; Orenstein *et al.*, 1997).

The duration of the training period seems to be a crucial factor when the main goal is modulation of autonomic control of the cardiovascular system, both in its tonic and phasic components. Thus, training affected only the reflex activity of the autonomic nervous system in patients underwent 4 weeks of rehabilitation after a first myocardial infarction (La Rovere *et al.*, 1992), whereas up to 6 months of regular physical exercise were required to obtain a significant increase in 24-hour parasympathetic tone in patients with left ventricular dysfunction and low HRV after infarction (Mazzuero *et al.*, 1994). A moderate long period of endurance training can, therefore, modulate the cardiovascular autonomic control of heart rate in a manner similar to that obtained with ß-blockers (Sandrone *et al.*, 1994) or with muscarinic receptor stimulation (De Ferrari *et al.*, 1993), which has been associated with favourable prognosis. Rest had traditionally been recommended for all patients with CHF until the late 1980s (Smith *et al.*, 1988). Several investigators demonstrated that patients with impaired left ventricular function could obtain some benefit from cardiac rehabilitation programmes without any detectable deterioration in left ventricular ejection fraction (Lee *et*

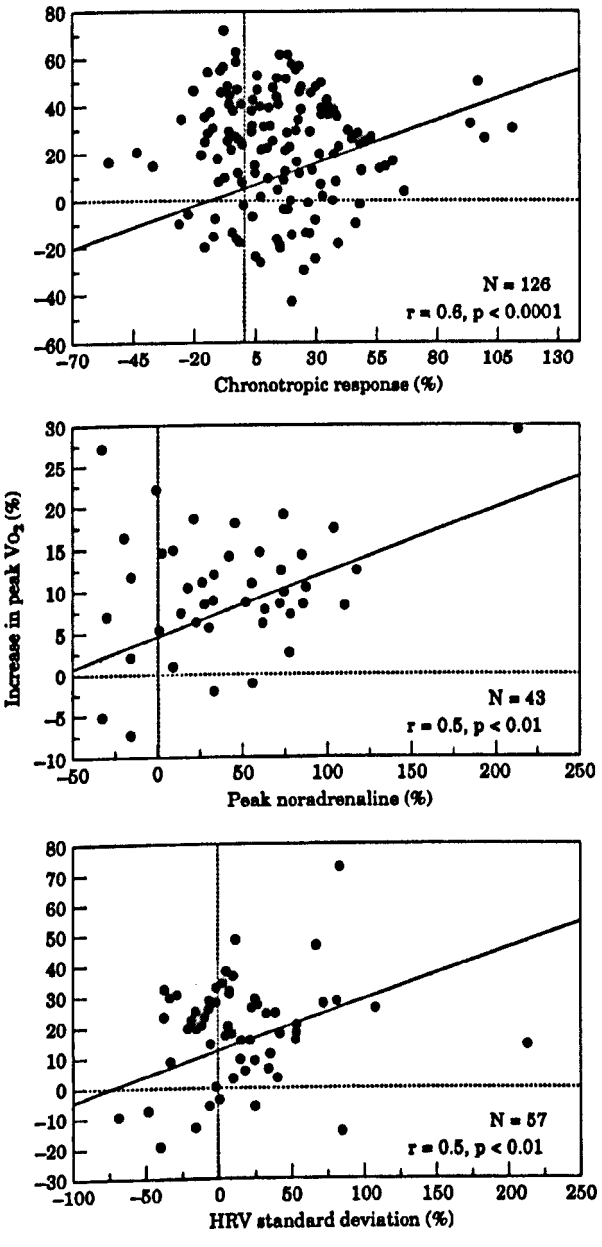

Figure 1.9. Correlations between measurements of autonomic balance and improved exercise performance with physical training in stable heart failure patients. Significant relations are demonstrated between training-induced increase in peak oxygen uptake (VO_2max) and training-induced percentage changes in chronotropic responsivenes, plasma noradrenaline at peak exercise and standard deviation of heart rate variability (HRV) (adapted from European Heart Failure Training Group, 1998).

al., 1979; Conn *et al.*, 1982) or additional negative effect on infarct expansion (Froelicher *et al.*, 1984). It was not until the end of the 1980s that the first uncontrolled reports were published of patients with CHF achieving increases in exercise performance after physical training (Sullivan *et al.*, 1988a and 1989a). Although this has now been confirmed in controlled trials in heart failure (Coats *et al.*, 1990a), very few controlled studies have been conducted to look at the effects of physical training programmes on the autonomic regulation of the cardiovascular function in this category of patients (Coats *et al.*, 1992). Recently, reversal of autonomic derangements, by increasing the parasympathetically mediated component of HRV, has been shown with physical training in patients with CHF (Kiilavuori *et al.*, 1995). Even more recently, the European Heart Failure Training Group (1998) reviewed the progress of 134 stable heart failure patients, studied in randomised controlled trials of physical training, and reported a good correlation between training-induced beneficial changes in autonomic parameters (chronotropic responsiveness, NA at peak exercise and standard deviation of HRV) and improvement in exercise performance (Figure 1.9). Abnormalities of muscle afferents are hypothesised to underlie the neurohormonal activation in heart failure and physical training not only improved the exercise capacity of the trained forearm but also partially reversed the abnormal sympathetic, vasoconstrictor and ventilatory responses to exercise via a reduction of the muscle ergoreflex excitation (Piepoli *et al.*, 1996).

Chapter II

Methodological Aspects

A. Phosphorus-31 Magnetic Resonance Spectroscopy (^{31}P MRS)

Muscle metabolism is traditionally measured via biochemical analysis of tissue biopsies, which is a technique with significant limitations regarding repeatability and feasibility in the in vivo situation. Phosphorus-31 MRS provides the opportunity of a serial non-invasive assessment of inorganic phosphate (Pi), phosphocreatine (PCr) and ATP levels and intracellular pH (Figure 2.1), all indices of glycolytic activity and mitochondrial respiratory control, both at rest and during exercise (Chance *et al.*, 1981; Taylor *et al.*, 1983). Phosphorus-31 MRS studies of skeletal muscle metabolism in heart failure have shown increased PCr breakdown and intracellular acidosis during exercise, both in human subjects (Wilson *et al.*, 1985c; Wiener *et al.*, 1986; Massie *et al.*, 1987 a&b; Mancini *et al.*, 1988; Ragagopalan *et al.*, 1988; Arnolda *et al.*, 1990; Stratton *et al.*, 1994) as well as in rats following a myocardial infarct (Arnolda *et al.*, 1991). This increase in PCr breakdown and intracellular acidosis implies an increased glycolytic contribution to the required ATP synthesis, due either to an increase in the requirements for ATP (resulting perhaps from muscle atrophy or decrease in metabolic efficiency), to a defect in oxidative ATP synthesis, or to a primary alteration in the balance between glycogenolytic and oxidative ATP synthesis. In our studies we examined a) the gastrocnemius muscle at rest and during exercise in patients with CHF and in healthy control subjects to look at the effects of physical training on skeletal muscle metabolism in heart failure, b) the dominant forearm muscle at rest and during exercise in patients with extensive anterior myocardial infarction to describe the time course of skeletal muscle

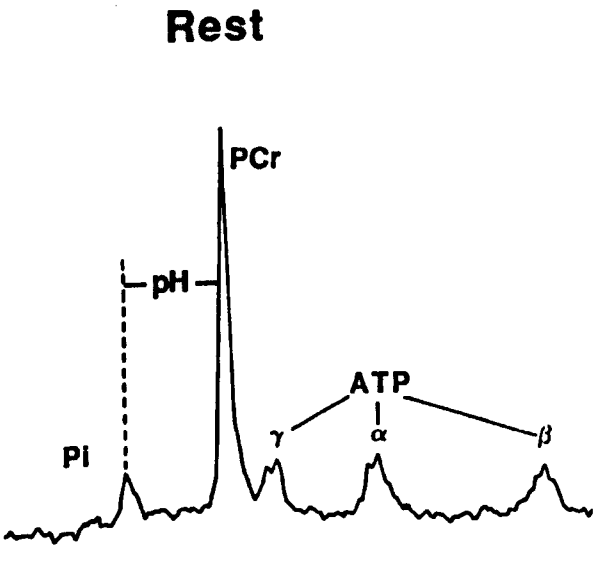

Figure 2.1. Magnetic resonance spectroscopy signal allowing serial non-invasive assessment of inorganic phosphate (Pi), phosphocreatine (PCr), ATP levels and intramuscular pH. Resting 256-scan spectrum (~4 min) from forearm; Pi, PCr and the α, β, and γ components of ATP are shown. Intracellular pH is calculated from chemical shift difference between Pi and PCr peaks.

metabolism following first large anterior myocardial infarction and c) the calf muscles during sciatic nerve stimulation at 2 Hz in a rat model with myocardial infarction to study the influence of exercise training on muscle metabolism in experimental heart failure. We also analysed the PCr recovery following exercise, which has been proposed as a measure of muscle oxidative capacity that is independent of muscle mass, recruitment and workload (Drexler *et al.*, 1992; Mancini *et al.*, 1992b).

In the study of skeletal muscle bioenergetics three major problems arise: a) The first is with the measurements themselves: much effort and ingenuity have been devoted to the acquisition, localisation and quantification of the MRS signal and the calculation of metabolite concentrations. Technical and methodological advances, however, allow reproducible MRS-derived parameters assessing muscle aerobic and anaerobic metabolism (Thompson *et al.*, 1996). Moreover, the sinificant correlation between mitochondrial oxidative enzyme citrate synthase and PCr utilisation in experimental heart failure (Arnolda *et al.*, 1991), indicate that MRS changes reflect abnormalities in oxidative capacity of skeletal muscle and may contribute to better understanding of the pathophysiological basis of limiting symptoms in CHF. b) The second problem is the experimental design: the scope of skeletal muscle studies is considerably widened by analysis of exercise and recovery, during which ATP turnover and proton fluxes vary greatly and many stimulation and voluntary exercise protocols have been devised for different purposes. c) The third problem is how to go beyond qualitative inference to estimate rates of aerobic and glycogenolytic ATP synthesis and proton efflux and to study their relationship to the concentrations of their putative regulators (i.e. from measurements of pH and metabolite concentrations). Quantitative bioenergetic interpretation of skeletal muscle ^{31}P MRS will become more reliable and informative as our knowledge of these processes improves.

For studies of *flexor digitorum superficialis* muscle, the dominant arm was placed in a 1.9 Tesla (T), 20 cm bore superconducting magnet (Oxford Instruments, Oxford, U.K.) interfaced to a Biospec spectrometer (Oxford Research Systems, Oxford, U.K.) and a 2.5 cm diameter surface coil was placed over the muscle. Prior to testing, the body of each flexor digitorum superficialis was identified and an approximate 2 cm diameter circular area marked with a pen to position the surface coil. Reproducible surface coil positioning between studies was accomplished as follows: a line was drawn between bony landmarks on the olecranon process and the distal ulna; the distance from the olecranon to centre of the circle was noted; additionally, the distance from the bottom of the circle to the line was noted. After magnetic field homogeneity had been optimised using the proton signal, ^{31}P spectra were collected at rest, during exercise and during recovery. Spectra were acquired using a 2-sec interpulse delay at rest (128 scans) and during finger flexion (32 scans) at a power output of 0.25-W for 4 spectra, incremented by 0.08-W for each of the remaining spectra. Exercise was continued until fatigue. The muscle was then studied for 12 min during recovery (four 16-scan spectra, four 32-scan spectra and then two 64-scan spectra) (Stratton *et al.*, 1994).

For studies of *gastrocnemius* muscle, subjects were placed in a 1.9 T 60 cm bore superconducting magnet (Oxford Magnet Technology, Eynsham, Oxford, U.K.) interfaced to a Bruker spectrometer (Bruker, Coventry, U.K.) with the right calf overlying a 6.0 cm diameter surface coil. Magnetic field homogeneity was adjusted by using the proton free induction decay until the linewidth of the water resonance at half-height was 40 Hz or better. Phosphorus spectra were acquired at 32.7 MHz using a 2-sec interpulse delay at rest (128 scans) and during plantar flexion (32 scans) at a power output of 1.5-W for 4 spectra, incremented by 0.5-W for each of the remaining spectra. Exercise was continued until fatigue and therefore the number of exercise spectra collected varied according to the exercise capacity of the subject. The muscle was then studied for 12 min during recovery. Four spectra each of 16 scans (32 sec per spectrum) were collected on cessation of the exercise. Thereafter, 32 scans were summed for each of eight spectra (64 sec per spectrum) (Adamopoulos *et al.*, 1993).

For studies of *calf muscle* in the rat model of experimental heart failure sciatic nerve stimulation at 2 Hz was used to cause contraction of the plantaris, gastrocnemius and soleus muscle as a group (distal hindlimb). All experiments were performed at a frequency of 73.8 MHz for ^{31}P in a vertical wide bore 4.3 T magnet. The radiofrequency coil was a Dadok coil surrounding the plantaris-gastrocnemius-soleus group of muscles of the rat leg. The same coil was used for both transmission and reception. Free induction decays (FIDs) were collected as 2K data with a sweep width of 2000 Hz. The study was done using a tip angle of 60° and 2-sec interpulse delay. A first acquisition of 32 pulses (80 sec) was obtained at rest. Then the sciatic nerve was stimulated for 640 sec at the maximum obtainable twitch tension. This permitted aquisition of 8x32 pulse spectra from the muscle during exercise and then a 640 sec recovery was allowed during which a further 4x8 and then 7x32 pulse spectra were collected. The 32-pulse spectrum acquired at the end of the recovery period was used to assess the completeness of the recovery of the muscle indicated. At this time PCr recovery was complete and Pi was frequently undetectable. The sciatic nerve was again stimulated during 320 sec with a calibrated submaximal sciatic nerve stimulation allowing a twitch tension of 200 g in order to normalise the twitch tension in all the animals studied. We added this additional stimulation protocol in our animals to avoid the variation in tension produced in the maximal stimulation protocol resulting from changes in local collection of fluid around the nerve and the electrodes (Brunotte *et al.*, 1995).

Relative concentrations of Pi, PCr and ATP were obtained by manual triangulation of peak areas and were corrected for magnetic saturation. Absolute concentrations of PCr and Pi were obtained assuming a normal ATP concentration of 8.2 mM (mM, that is mmol/l of muscle cytosolic water) (Arnold *et al.*, 1984). During exercise it is convenient to express PCr concentration as PCr/(PCr+Pi) to allow for changes in signal intensity due to possible movement. Intracellular pH was calculated from the chemical shift of the Pi peak, relative to PCr (measured in ppm), as $pH = 6.75 + \log_{10}\{(ppm - 3.27)/ (5.69 - ppm)\}$.

Free cytosolic ADP concentration [ADP] was calculated from pH and PCr concentration using the creatine kinase equilibrium constant (Veech *et al.*, 1979) of $K_{eq} = 1.66 \times 10^9\ M^{-1}$,

assuming a normal total creatine (TCr) content (Arnold *et al.*, 1984) of 42.5 mM, as [ADP] = ([TCr]/[PCr] - 1)x[ATP]/($K_{eq}[H^+]$). Note that there was no significant alteration in concentrations of ATP or total creatine in a rat model of severe heart failure (Arnolda *et al.*, 1991; Thompson *et al.*, 1994). The kinetic analysis of the MRS data from exercise and recovery is outlined below.

i. Analysis of Recovery

During recovery, PCr is resynthesised as a consequence of oxidative ATP synthesis, and this is accompanied by the net generation of protons which are lost from the cell by several processes with an approximately linear pH-dependence (Kemp *et al.*, 1994 b&c). Thus, by analysing recovery (which is metabolically simpler than exercise) we can estimate rates of mitochondrial ATP synthesis and net proton efflux and so infer the capacity of the cell to perform both processes.

Analysis, therefore, of recovery yields information about mitochondrial function. The initial rate of PCr resynthesis is an estimate of the end-exercise rate of oxidative ATP synthesis (Boska, 1991; Kemp *et al.*, 1994b) and has a hyperbolic (Michaelis-Menten) relationship to its driving force, the cytosolic free ADP concentration (Kemp *et al.*, 1993a). Thus, the end-exercise [ADP] and initial PCr resynthesis rate (V) can be used to calculate the *maximum rate of oxidative ATP synthesis* (Q_{max}) (Kemp *et al.*, 1993c), which is a quantitative measure of mitochondrial capacity (a function of mitochondrial content, mitochondrial activation state and blood flow). The maximum rate of oxidative ATP synthesis by the muscle (Q_{max}) is provided by the following equation: $Q_{max}=V\{1+K_m/[ADP]\}$ assuming the normal value of K_m=30μM (Kemp *et al.*, 1993c). Another inverse measure of mitochondrial function (Kemp and Radda, 1994a), the half-time of PCr recovery, is calculated from the slope of a semilogarithmic plot (Arnold *et al.*, 1984). For analysis of PCr recovery, the end of the last spectral collection during exercise was defined as t = 0. The initial rate of PCr recovery was calculated as the slope of a regression line fitted to the values of PCr concentration in the end-exercise spectrum (t = 0) and in the first two recovery spectra (midpoints at t = 0.27 and 0.83 min). For this analysis of recovery, the decrease in PCr during the acquisition of the last exercise spectrum was corrected for by linear extrapolation of PCr/(PCr+Pi) from the midpoints of the last two exercise spectra (t = -1.75 and -0.5 min) to the end of the last spectral collection (t = 0).

Analysis of recovery also yields information about proton efflux. Measurements of pH and PCr concentration are used to calculate the *initial recovery proton efflux rate* (Kemp *et al.*, 1993b and 1994b) (i.e. the rate by which protons 'disappear' from the cytosol during the initial stage of recovery) and from this a first order *proton efflux rate constant* (Kemp *et al.*, 1992). A modification to this calculates the rate of end-exercise proton efflux required to make the calculated end-exercise rate of oxidative ATP synthesis equal to the initial post-exercise rate of PCr resynthesis. In gastrocnemius, pH changes are smaller than in forearm muscle, which makes the proton efflux correction less important and we, therefore, apply a

small correction based on the average rate of pH and PCr concentration and the mean value of the proton efflux rate constant in patients and controls.

ii. Analysis of Exercise

Exercise differs from recovery in that glycogenolysis occurs and mechanical work is done. During exercise, ATP is produced by net hydrolysis of PCr, by glycogenolysis to lactic acid and by oxidative phosphorylation; protons are produced by glycogenolysis, buffered by passive processes and as a consequence of PCr breakdown, and also leave the cell by several membrane transport processes (Kemp *et al.*, 1993b, 1994 a&b). Net PCr hydrolysis, and therefore ATP supplied, is measured directly. Measurements of pH and PCr concentration are used to calculate the rate at which protons are buffered. With appropriate correction for net proton efflux, this estimates lactic acid production, which produces 1.5 ATP per proton (i.e. glycogenolytic ATP synthesis) (Kemp *et al.*, 1993b, 1994 a&b).

Measurements of pH and PCr concentration from rest to the first exercise spectrum are used to calculate the initial rate of non-oxidative ATP synthesis (Kemp *et al.*, 1993b and 1994b). We, thus, calculate the rate of glycogenolysis to lactic acid and so the initial rate of glyocogenolytic ATP synthesis. The early contribution of oxidation can be neglected (Kemp *et al.*, 1994c) and so the sum of glyocogenolytic ATP synthesis rate and the initial rate of PCr depletion gives the *initial rate of ATP turnover*, which is equivalent, in practice, to the initial ATPase rate measured by the very early rate of PCr depletion (Foley and Meyer, 1993). For a given initial power output, the *initial rate of ATP turnover* is inversely proportional to muscle mass and to metabolic efficiency, and for present purposes we take these together as the *effective muscle mass* (EMM).

Subsequent changes in pH and PCr concentration during exercise are used to calculate the rate of glycogenolytic ATP synthesis at each point, allowing for proton efflux by using the proton efflux rate constant (i.e. its slope against pH). The *total ATP synthesis rate* at each point is estimated using the initial ATP synthesis rate and the mechanical power output. *Total ATP turnover rate* in the later stages of exercise is estimated as the product of the *initial ATP turnover rate* and the ratio of the mechanical power output at each point to the mechanical power at the beginning of exercise. From the *total ATP turnover rate* and the rates of glycogenolysis and PCr depletion, the *oxidative ATP synthesis rate* is calculated by difference (Kemp *et al.*, 1993b, 1994 a&b).

At any point during exercise, the ADP concentration and the oxidative ATP synthesis rate are used to calculate the time-course of *apparent* Q_{max} (the value of Q_{max} calculated from the end-exercise ADP concentration and initial PCr resynthesis rate -see above- is a special case of this). While the true maximum rate of oxidative ATP synthesis is a function only of mitochondrial density and intrinsic mitochondrial performance (Kemp *et al.*, 1993c), apparent Q_{max} during exercise reflects also the changes in vascular oxygen delivery during exercise.

To facilitate intersubject comparisons where exercise durations differ widely, it is convenient to calculate total ATP synthesis over the whole of exercise, and express this relative to total work done (Kemp *et al.*, 1995). Calculating, thus, separately the overall oxidative and non-oxidative ATP costs of work distinguishes between the effects of reduced effective muscle mass and of reduced Q_{max} (i.e. reduced mitochondrial capacity) on the changes in exercise. The *overall non-oxidative cost of work* will tend to be increased both by a pathological fall in the oxidative contribution (so the non-oxidative contribution must rise) and by a decrease in effective muscle mass (which requires increased ATP supply by all means). Similarly, the *overall oxidative cost of work* will tend to be decreased by a fall in the oxidative contribution to exercise, but increased by a fall in effective muscle mass. Thus, falls in effective muscle mass and oxidative contribution have the same effects on the non-oxidative cost of work, but opposite effects on the oxidative cost of work.

These effects can be separated by using the initial rate of ATP turnover to estimate work per unit effective muscle mass, and using this to calculate the cost of work per unit effective muscle mass. The *non-oxidative cost of work per unit* effective muscle mass will be increased by any defect in the oxidative contribution to exercise, while the *oxidative cost of work per unit effective muscle mass* will be reduced by an oxidative defect (Kemp *et al.*, 1995). Both quantities are independent of effective muscle mass.

B. Heart Rate Variability (HRV) Assessment

The clinical importance of HRV was first recognised in 1965 by Hon and Lee (1965) who noted that fetal distress was preceded by alterations in interbeat intervals. During the 1970's a number of simple bedside tests based on short-term R-R differences was introduced by Ewing *et al.* (1985) to assess autonomic neuropathy in diabetic patients. The first observation that HRV could be used as a predictor of post-infarction mortality was published in 1978 (Wolf *et al.*, 1978). In 1981, Akselrod *et al.*, introduced power spectral analysis, a mathematical analysis of heart rate fluctuations around the mean to quantitatively evaluate beat-to-beat autonomic control of the cardiovascular system.

i. Time Domain

The simplest methods to evaluate the variations in heart rate are the time domain measures. Despite their simplicity, these measurements, the most common of which is standard deviation (SD), proved to be useful clinical tools. In a continuous ECG record, each QRS complex is detected and the so-called normal-to-normal (NN) intervals (that is, all intervals between adjacent QRS complexes resulting from sinus node depolarisations) are determined to estimate the SD of all normal R-R intervals (SDNN) for a given period of recording. The practical use of HRV in the time domain in adult medicine has been reached mainly in two clinical scenarios: reduced SDNN can be used as a predictor of outcome in patients who had survived an acute myocardial infarction (Kleiger *et al.*, 1987) and as an early warning sign of diabetic neuropathy in diabetic patients without the usual

cardiovascular signs of autonomic neuropathy (Murray *et al.*, 1975). Depressed HRV is not only a powerful predictor of total cardiac mortality after myocardial infarction but also identifies patients at risk of sudden death or sustained symptomatic ventricular tachycardia (Odemuyiwa *et al.*, 1991) better than left ventricular ejection fraction. Moreover, investigating separately 'parasympathetic' (SDNN, rMSSD, sNN50) and 'sympathetic' (SDANN, SDNN index) time domain HRV variables Bigger *et al.* (1988) showed significantly reduced parasympathetic activity in patients at risk of mortality after myocardial infarction. The predictive value of HRV measures is independent of other factors established for postinfarction risk stratification, such as reduced left ventricular ejection fraction, increased ventricular ectopic activity and presence of late potentials.

Recent investigations have established that analysis of HRV in the time domain (SDNN <100 msec) can also identify patients with ischaemic or idiopathic dilated cardiomyopathy who have an increased risk of cardiac death or heart transplantation (Ponikowski *et al.*, 1997a; Fauchier *et al.*, 1997). Whether analysis of HRV in the time domain could be recommended in the risk stratification for better management of patients with CHF needs further investigation. A more recent, large, prospective study suggests that a reduction in SDNN (<100 msec) identifies ambulant patients with CHF at high risk of death and represents a better predictor of death due to progressive heart failure than other conventional clinical measurements (Nolan *et al.*, 1998). Authors further propose that high-risk subgroups identified by this measurement should be candidates for additional therapy for CHF patients already treated with an ACE inhibitor. The SDNN index is modulated by multiple mechanisms and a low SDNN in CHF reflects the presence of a major degree of physiological dysfunction consisting of abnormalities in sympathetic, parasympathetic and RAA systems, abnormal chemoreceptor function, changes in respiratory pattern and physical deconditioning (Malliani *et al.*, 1991; Bernardi *et al.*, 1996; Ponikowski *et al.*, 1996; Mortara *et al.*, 1997b).

Although optimised cutoff values defining normal and depressed HRV have not been accurately established, several independent prospective studies indicate that cutoff values of 24-hour SDNN <50msec and HRV triangular index <15 for highly depressed HRV or SDNN <100msec and HRV triangular index <20 for moderately depressed HRV are likely to be broadly applicable (Task Force, 1996). Depressed SDNN has been interpreted as a sign of decreased parasympathetic activity, although its value as a measure of changes in sympatho-vagal balance is considered rather limited. The SDNN index is modulated predominantly by low-frequency cyclical changes reflecting thermoregulatory mechanisms, fluctuations in activity of the RAA system, the function of peripheral chemoreceptors, changes in the respiratory pattern and physical activity (Malliani *et al.*, 1991; Parati *et al.*, 1995; Bernardi *et al.*, 1996; Mortara *et al.*, 1997b).

The following four measures are recommended for time domain assessment of HRV: a) SDNN, standard deviation of all normal R-R intervals (estimate of overall HRV), b) Heart rate variability triangular index, the integral of the density distribution (that is, the number of all R-R intervals) divided by the maximum of the density distribution (an estimate of overall

HRV), c) SDANN, the standard deviation of the average R-R intervals calculated in all 5-min segments of the entire recording (estimate of long-term components of HRV) and d) rMSSD, the square root of the mean squared differences of successive R-R intervals (estimate of short-term components of HRV).

ii. Frequency Domain

Various spectral methods for the analysis of the tachogram have been applied since the late 1960s (Kay and Marple, 1981). Power spectral analysis provides the basic information of how power (variance) distributes as a function of frequency. Independent of the method used (non-parametric or parametric) only an estimate of the true power spectral density of the signal can be obtained by proper mathematical algorithms. Most studies have relied either on fast Fourier transformation (FFT) (Kitney and Rompelman, 1980; Akselrod *et al.*, 1981) or on the autoregressive algorithm (Pagani *et al.*, 1986) to assess the number, centre frequency and amplitude of the oscillatory components hidden in the HRV signal. The advantages of the non-parametric methods are a) the simplicity of the algorithm used (FFT in most of the cases) and b) the high processing speed while the major disadvantage is that FFT requires a priori selection of the number and frequency range of the bands of interest. The advantages of the parametric methods are a) automatic calculation of the number, centre frequency and associated power of oscillatory components (low- and high-frequency power) without the need for a priori decisions, b) smoother spectral components that can be distinguished independent of pre-selected frequency bands and c) an efficient estimation of power spectral density even on short segments of data (for example 200 cycles rather than the more usual 512 cycles) which are more likely to be stationary and easier to obtain during clinical studies, given that strictly speaking stationary conditions are unknown in biological systems (Malliani *et al.*, 1994). The main disadvantage of parametric methods is the need of verification of the suitability of the chosen model (i.e. the order of the model).

Three major spectral components are recognised in a spectrum calculated from short-term recordings (up to 5 min): VLF (very low-frequency), LF and HF components, whereas when analysis of the sequence of R-R intervals of longer periods (usually 24-hour period) is performed the result then includes an ULF (ultra low-frequency) component in addition to VLF, LF and HF components. The distribution of the power and the central frequency are not fixed but vary within the range of ≤0.04 Hz for VLF, 0.04-0.15 Hz for LF and 0.15-0.4 Hz for HF from short-term recordings as well as ≤0.003 Hz for ULF, 0.003-0.04 Hz for VLF, 0.04-0.15 Hz for LF and 0.15-0.4 Hz when analysis of entire 24 hours is performed. The power of the HF and LF spectral components of HRV can be expressed as normalised units (nu), representing the relative percentage of each spectral component compared with the total oscillatory power, or as absolute ($msec^2$). The normalisation procedure has proved essential for the interpretation of data (Pagani *et al.*, 1986).

The HF component is entrained by the respiratory frequency and, apart from a small residual (probably mechanical) respiratory component seen after cardiac transplantation (Bernardi *et al.*, 1989), is carried almost entirely by vagal activity. Disagreement exists in respect to the LF component. The LF rhythm is mediated jointly by the vagal and sympathetic nervous system (Pomeranz *et al.*, 1985; Appel *et al.*, 1989) but predominantly by the latter, especially when expressed in normalised units (Pagani *et al.*, 1986; Malliani *et*

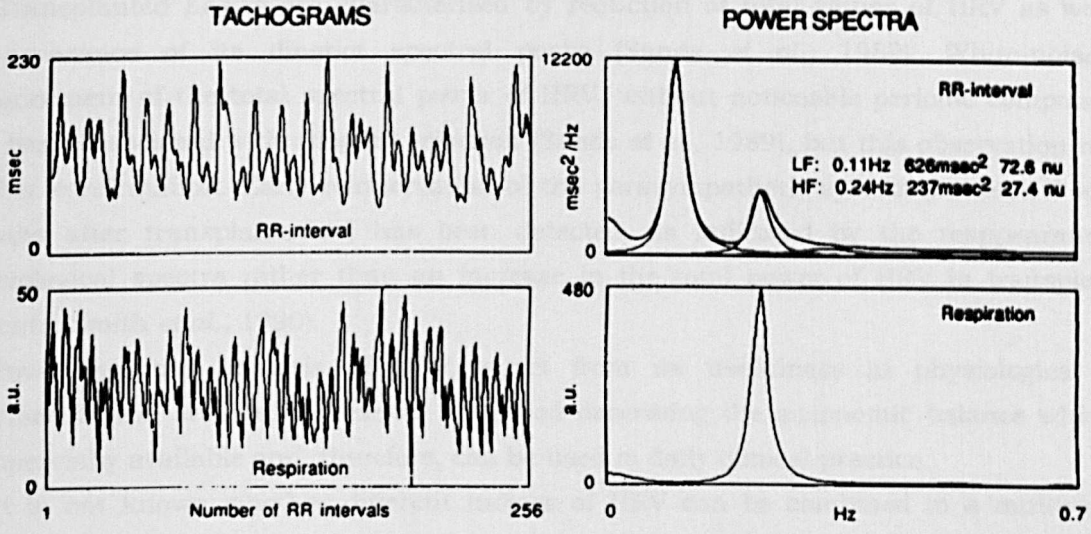

Figure 2.2. Power spectral analysis of heart rate variability. Tracings show the R-R interval tachogram (display of individual R-R intervals) for 256 consecutive beats (upper left panel) and the low-(0.11 Hz) and high-frequency (0.24 Hz) spectral components after power spectral analysis of R-R variability (upper right panel) in a normal subject. Lower panels represent spectral analysis of the respiratory trace, performed on the signal sampled once every cardiac cycle. These spectra are used as a reference to identify the frequency of R-R oscillations caused by respiratory sinus arrhythmia. The high-frequency (HF) peak corresponds to respiratory sinus arrhythmia (lower right panel) and reflects the cardiac vagal activity, whereas the low-frequency (LF) peak can be mediated by both sympathetic and vagal outflow to the heart.

al., 1991) (Figure 2.2). Consequently, the LF/HF ratio is considered by some investigators to mirror sympatho-vagal balance (reflecting both sympathetic and vagal activity) or by others to reflect only the sympathetic modulations (Task Force, 1996). Physiological interpretation of lower frequency components of HRV (VLF and ULF) warrants further elucidation.

Recent investigations have also suggested a potential role of frequency domain measures of HRV to predict both death and arrhythmic mechanisms early and late after myocardial infarction (Farrel *et al.*, 1991; Bigger *et al.*, 1992a and 1993). Spectral analysis of HRV in survivors of myocardial infarction suggested that the ULF and VLF components carry the highest predictive value (Bigger *et al.*, 1992b).

Heart failure is characterised by a shift in sympatho-vagal balance towards parasympathetic withdrawal and sympathetic predominance, expressed by reduced total power and diminished LF and HF components of HRV, although the LF/HF ratio becomes higher in moderate to severe CHF. The biological relevance, however, of spectral analysis of HRV is severely limited when variance is restricted in conditions such as advanced heart

failure or complicated myocardial infarction. Advanced heart failure is likely to be accompanied by decreased responsiveness of sinus node to neural modulation (due to excessive sympathetic activation) and therefore the extremely low variance, manifested with nearly absent LF component of HRV, would not reflect this considerable increase in sympatho-excitation. In contrast, diabetic neuropathy is associated with abnormally reduced total power but with unchanged LF/HF ratio suggesting a simultaneous effect on both sympathetic and vagal modulations (Malliani *et al.*, 1994).

Transplanted hearts are characterised by reduction of total values of HRV as well as disappearance of its dinstict spectral peaks (Sands *et al.*, 1989). White-noise-like enhancement of the total spectral power of HRV, without noticeable periodic components, has been associated with allograft rejection (Sands *et al.*, 1989), but this observation needs further confirmation. Early reinnervation of the parasympathetic system (between 3 and 6 months after transplantation) has been detected, as indicated by the reappearance of physiological spectra rather than an increase in the total power of HRV in transplanted patients (Smith *et al.*, 1990).

Power spectral analysis of HRV, apart from its usefulness in physiological and pharmacological studies, represents a method describing the autonomic balance which is commercially available and, therefore, can be used in daily clinical practice.

It is not known whether different indices of HRV can be combined in a multivariate fashion in order to improve postinfarction risk stratification or prediction of progress and final outcome in the syndrome of CHF. No currently recognised measure, however, provides better prognostic information than the time domain indices assessing overall HRV (SDNN or HRV triangular index), with the exception, probably, of the ULF component of the entire 24-hour spectral analysis, which performs equally well (Task Force, 1996).

C. Radiolabeled Noradrenaline (NA) Kinetics

The relationship that usually exists between sympathetic nerve firing and NA synthesis and release rates (Yamaguchi *et al.*, 1975) provides the experimental justification for the use of NA turnover measurements to assess sympathetic nervous activity. Noradrenaline in plasma is derived largely from transmitter released by sympathetic nerves, with only a small contribution from the adrenal medulla and apparently no input at all from the central nervous system (Hoeldtke *et al.*, 1974; Silverberg *et al.*, 1978). The lack of a completely acceptable compartmental model to determine whole-body NA kinetics, has forced the investigators to turn to the plasma concentration of the transmitter as an index of overall sympathetic nervous system activity (Cryer, 1980). The dependence, though, of the plasma NA concentration on the rate at which NA is removed from the circulation and not solely on the rate of NA release represents the main objection to this application of plasma NA values. To avoid this confounding influence of NA plasma clearance, which is reduced in severe CHF as a consequence of impaired visceral blood flow, metabolic clearance rate methods have

been developed, based on NA isotope dilution in plasma, to measure the rate of spillover (appearance) of NA to plasma.

Recently developed radiotracer kinetic techniques, using infusions of [^{3}H]NA of high specific activity, can assess NA spillover to plasma and NA plasma clearance simultaneously (Esler *et al.*, 1984). Whole-body NA spillover (or NA apparent release rate) is the overall rate at which NA enters plasma and whole-body NA clearance is the volume of plasma cleared of NA by the body per unit time. These radiotracer techniques assume that a steady state has been reached, so that infused [^{3}H]NA is not released in a significant degree and that alumina extractable [^{3}H] metabolites do not accumulate during [^{3}H]NA infusion. Several studies have demonstrated that these assumptions are valid (Esler *et al.*, 1979; McCance and Forfar, 1988 and 1989a).

Rather than the rate of release of NA from sympathetic nerve varicosities, NA spillover rate expresses the rate at which NA released enters plasma. In humans this rate appears to be approximately 20% of the total turnover of NA in sympathetic nerves (Hoeldtke *et al.*, 1983). Although this technique estimates NA spillover to plasma and not NA release from nerve endings, it has been shown for several organs, including the heart (Blombery *et al.*, 1983), that the regional NA spillover correlates well with the rate of direct sympathetic nerve stimulation (Hasking *et al.*, 1986).

The within day coefficient of variation is 4% for the estimation of plasma NA and 7% for [^{3}H]NA. Each sample was assayed in duplicate, with half of each alumina extract being used for estimation of [^{3}H]NA and half for estimation of NA. Noradrenaline plasma clearance and whole-body NA spillover were measured according to the following relations, which hold under steady state conditions (Hasking *et al.*, 1986)

$$\text{Whole-body NA clearance} = \frac{\text{Infusion rate (dpm/min)}}{\text{Plasma [}^3\text{H]NA (dpm/ml)}}$$

$$\text{Whole-body NA spillover} = \frac{\text{Infusion rate (dpm/min)}}{\text{Specific radioactivity of plasma NA (dpm/pg)}}$$

where dpm is disintegrations per minute of tritiated NA.

It has been shown that after the attainment of steady-state conditions at rest, the plasma concentration of endogenous and tritiated NA are in a new steady-state condition after 5 min of exercise (McCance and Forfar, 1989a)

The mean concentrations in two to four arterial blood samples were used to calculate total NA specific activity, spillover and clearance.

Assessment of human sympathetic nervous activity from measurements of NA turnover constitute an improvement on the previously used static measurements of NA concentration in urine or plasma. Thus, total NA spillover is lowered in the presence of reduced

sympathetic nerve activity, whether from sympathetic nerve failure (idiopathic peripheral autonomic insufficiency), due to dosing with the sympathetic nervous system suppressant clonidine or accompanying physical conditioning, whereas increases in NA release are observed in several disease states, including heart failure, cirrhosis, depressive illness and essential hypertension (Esler *et al.*, 1988).

Numerous studies with steady state infusions (isotope dilution) of tritiated NA in patients with moderate or severe CHF have demonstrated enhanced overall and regional NA spillover from the heart and kidneys (Hasking *et al.*,1986; Meredith *et al.*, 1993; Kaye *et al.*, 1994; Eisenhofer *et al.*, 1996). The elevated cardiac NA spillover results from the combination of increased neuronal release (compatible with augmented nerve firing) and impaired neuronal uptake of NA (Meredith *et al.*, 1993; Böhm *et al.*, 1995; Eisenhofer *et al.*, 1996), and is disproportionately higher compared with that of other organs (Meredith *et al.*, 1993; Kaye *et al.*, 1994). In patients with mild-to-moderate CHF, however, a selective increase in cardiac adrenergic drive has been shown by using isotope dilution with steady state infusions of [^{3}H]NA, characterised by increased amounts of transmitter available at neuroeffector junctions for receptor binding, caused primarily by impaired NA uptake mechanisms (Rundqvist *et al.*, 1997). This increased cardiac adrenergic activity in these early stages of CHF appears to precede the augmented sympathetic outflow to the kidneys and skeletal muscle found in advanced CHF. Thus, cardiac, renal and total body NA spillover values in patients with severe CHF are high, as is sympathetic nerve activity to the skeletal muscle vascular bed in this patient category (Hasking *et al.*, 1986; Leimbach *et al.*, 1986; Rundqvist *et al.*, 1997), indicative of a more generalised hyperactivity of sympathetic discharge prevailing in advanced CHF.

Radiotracer NA kinetics provide useful information regarding the pathophysiological mechanisms by which a high cardiac adrenergic drive could contribute to worsening of cardiac function and progression of CHF. Hence, the increased concentrations of NA at cardiac receptor sites in both mild-to moderate and severe CHF may constitute the prerequisite for several secondary adverse effects for the failing heart, such as ß-receptor downregulation, pathological cardiac growth, depletion of NA stores and changes in regulatory G proteins (Bristow *et al.*, 1982; Neumann *et al.*, 1988). Furthemore, the documented association between cardiac NA spillover and malignant arrhythmias or myocardial ischaemia provide also important prognostic information (McCance and Forfar, 1989b; Meredith *et al.*, 1991; Kaye *et al.*, 1995). More specifically cardiac NA spillover values above the median of 310 pmol/min were found to be the most important predictible variable for death among patients awaiting cardiac transplantation (Kaye *et al.*, 1995).

Given that increased cardiac NA spillover in CHF has been associated with adverse prognosis and occurence of malignant ventricular arrhythmias (Meredith *et al.*, 1991; Kaye *et al.*, 1995), therapeutic interventions aiming at blocking this sympatho-excitation in patients with left ventricular dysfunction and mild symptoms might be beneficial. This important issue may be resolved in clinical trials with ß-blockers and other antagonists of the sympathetic nervous system (MERIT-HF and CIBIS-II studies, 1999).

Chapter III

Reproducibility of Heart Rate Variability Indices during Exercise Stress Testing and Inotrope Infusion in Chronic Heart Failure Patients

A. Abstract

Objectives. Heart rate variability represents a method describing the autonomic balance and apart from its usefulness in physiological and pharmacological studies can be also used in daily clinical practice. Little is known, however, about reproducibility of short-term sampling periods in patients with CHF both in static and during dynamic conditions.

Methods. Ten patients with CHF (aged 64±5 years, ejection fraction: 22±8%, peak oxygen uptake: 12.6±4 ml/kg/min) were studied to quantify the reproducibility of HRV from short term periods during stable conditions and during two different sympathetic stimulations: inotrope (dobutamine) infusion and physical exercise (supine bicycle exercise test) on two consecutive days at the same time of the day. Heart rate variability was assessed in the time (standard deviation of R-R intervals) and frequency (low- and high-frequency components) domain as absolute power (ln-msec^2) and as percentage of the total variability (normalised: nu).

Results. At rest the coefficient of variability (CV) of standard deviation was 16%, of low-frequency 10% and of high-frequency 14.5%. The HRV indices showed lower reproducibility during sympathetic compared with resting conditions, with CV varying from 4.1 to 31.7% for standard deviation, from 6.8 to 13.7% for low-frequency and from 14.1 to 25% for high-frequency component.

Conclusions. The reproducibility of HRV parameters is not particularly high, especially during dynamic conditions, and, therefore, a relative large day-to-day variations should be considered when HRV indices are used to assess changes in the autonomic control of the cardiovascular system.

B. Introduction

Spectral analysis of heart rate oscillations are increasingly studied not only as index of autonomic activity, but also because it conveys information about pathophysiological processes. This method has been extensively applied in normal subjects (Huikuri *et al.*, 1990; Kleiger *et al.*, 1991; Hohnloser *et al.*, 1992), and in patients with ischaemic heart disease (Kleiger *et al.*, 1987; Lombardi *et al.*, 1987; Bigger *et al.*, 1991), chronic congestive heart failure (Kienzle *et al.*, 1992; Stein *et al.*, 1995), and in patients with valvular heart disease (Stein *et al.*, 1993). The diurnal variations and reproducibility of HRV findings have been reported in normals from long recording periods (Hohnloser *et al.*, 1992; Huikuri *et al.*, 1990; Kleiger *et al.*, 1991), and also from short-term sampling periods (Freed *et al.*, 1994). Little is known about reproducibility of short-term periods in CHF subjects, both at rest and during dynamic conditions.

The purpose of this study was to quantify the reproducibility of parameters of HRV in CHF patients from short periods during stable conditions and during two different sympathetic stimulations: physical exercise and inotrope infusion.

C. Methods

i. Study Population and Design

Ten CHF patients (age: 64.3±5.4yrs; ejection fraction: 21.9±7.6%; peak oxygen uptake: 12.6±4.3ml/kg/min) gave informed consent for this study which was approved by the local ethics committee. Before the test, patients underwent a 2-4week familiarisation and baseline evaluation phase during which reproducible exercise and autonomic tests were obtained. Subsequently, the subjects underwent four dobutamine infusions and four supine bicycle exercise tests on a different day (2-30 days apart), at the same time of the day (09:00 hours), in a laboratory kept at 22-24°C.

Before the tests, the patients were fasting for at least three hours and took no tea, coffee or cigarettes and delayed taking their daily medications till after the test. On the days of exercise stress testing, the subjects were asked to pedal a supine bicycle ergometer, at the rate of 50 times/min, until exhaustion. The tests were performed in 5 min stages, with 25 Watt increments to the limit of tolerance while continuous records of heart rate and respiration were obtained. On the days of dobutamine infusion, while the subjects were maintaining a comfortable supine position, a dobutamine infusion was commenced by increasing infusion rates (5 µg/kg/min steps every 8 min) through a pump syringe till 80% maximal heart rate achieved, which was then maintained for a total infusion duration of 30 min. Heart rate and respiratory signals were recorded continuously throughout the infusion period.

ii. Power Spectral Analysis

Heart period and the respiratory signal were digitized on-line by a 12 bit analogue-to-digital converter (NB-MIO-16 board, National Instruments, Austin, Tx) at a sampling rate of 500 samples/sec. Power spectral analysis of heart periods and respiratory signals was performed by an autoregressive program (Bernardi *et al.*, 1990). The standard deviation (SD) of R-R intervals was calculated; the absolute powers of the harmonic components in the regions between 0.03 and 0.14 Hz (low-frequency component, LF) and between 0.15 and 0.40 Hz (high-frequency component, HF) were calculated after natural logarithm transformation (ln-msec^2), since preliminary tests had shown a skewed distribution. The relative predominance of each frequency component during exercise or dobutamine infusion (when the heart rate was increased and the HRV reduced) was computed by the relative power of each component as percentage of the total variability (normalised units: n.u.).

iii. Statistical Analysis

The reproducibility of the HRV variables was assessed by calculating: (1) the intraclass correlation coefficients of linear regression, r, calculated for each pair of HRV measurements; (2) the coefficient of variability (CV) i.e. the distribution of the differences between the readings obtained at the different phases of the study, as recommended by Bland and Altman (1986). Coefficient of variability was defined as the SD of the difference between paired measurements divided by the average value of the mean for each set of consecutive measurements. This coefficient represents 1 SD of variability, in percentage, relative to the mean of the sample.

D. Results

At rest before exercise or inotrope infusion, all the considered parameters showed not high reproducibility: the CV of SD was 0.4±4.6 msec (16%) (Figure 3.1), of LF 0.1±0.4 ln-msec^2 (10%), of HF 0.1±0.4 ln-msec^2 (14.5%). The HRV indices showed slightly lower reproducibility during sympathetic stimulations with respect to resting conditions. In fact the CV was varying for LF from 6.8% to 13.7% (fairly good reproducibility), from 4.1% to 31.7% for SD and from 14.1 to 25% for HF component. Overall the time domain measures of HRV (SD) were slightly less reproducible than the frequency domain.

Similar results were observed during inotrope infusion and during exercise (Table 3.1).

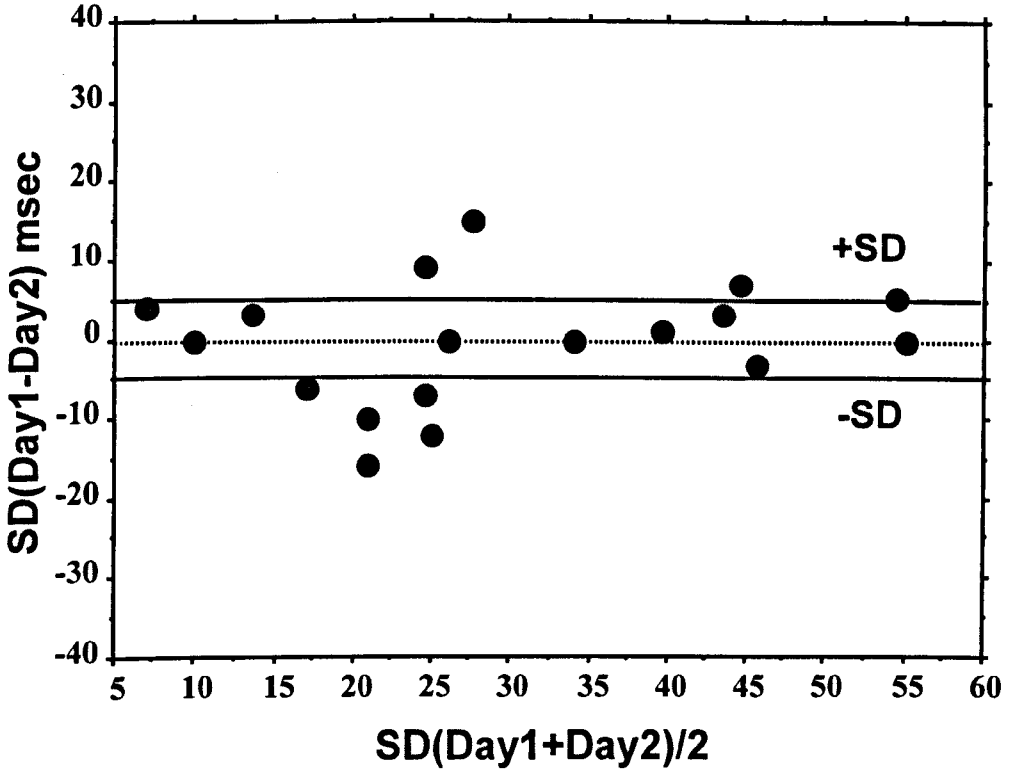

Figure 3.1. Bland Altman diagram showing the differences between Day 1 and Day 2 measurements of standard deviation (SD, vertical axis) and the mean differences between the two measurements (horizontal axis). The dotted line in the middle is the mean difference, while the continuous lines represent the ±standard deviations.

E. Discussion

A low reproducibility of HRV variables obtained from short-term sampling periods in static conditions has been previously observed. The result of the present study extends this finding also to CHF patients during dynamic conditions. In fact larger day-to-day variations of the various HRV measures were found: the variations ranged from 4% to approximately 30%. Similar observations have been reported by Hohnloser *et al.* (1992) who evaluated normals and patients with coronary artery disease and by Van Hoogenhuyze *et al.* (1991) who also examined the HRV of patients with CHF: however these groups analysed the reproducibility of HRV in long lasting Holter recordings. Stein *et al.* (1995), also evaluating 24-hour recordings, reported that in CHF patients the time domains indices had lower reproducibility with respect to frequency domain measurements (in agreement to our findings): however only for the very and the ultra low-frequency fluctuations the coefficient of correlations exceeded the value of 0.9. Instead Freed *et al.* (1994) analyzed short periods recordings in normals and ischaemic heart disease patients during controlled conditions:

TABLE 3.1. Intraclass Correlation Coefficients for the Measured Heart Rate Variability Variables During Various Study Conditions

Dobutamine infusion	**rest**	**db10**	**db15**	**db20**
SD	0.78±0.11	0.86±0.06	0.84±0.12	0.94±0.04
LF	0.79±0.14	0.80±0.15	0.87±0.04	0.91±0.05
HF	0.75±0.13	0.80±0.07	0.90±0.06	0.83±0.19
LF nu	0.84±0.06	0.73±0.13	0.70±0.10	0.61±0.20
HF nu	0.83±0.07	0.72±0.06	0.67±0.13	0.79±0.14
LF/HF	0.74±0.15	0.76±0.13	0.66±0.07	0.64±0.07
Supine exercise	**rest**	**25-W**	**50-W**	**75-W**
SD	0.77±0.09	0.80±0.12	0.87±0.05	0.71±0.17
LF	0.72±0.10	0.75±0.12	0.62±0.23	0.65±0.25
HF	0.89±0.03	0.67±0.13	0.80±0.10	0.83±0.19
LF nu	0.86±0.05	0.67±0.08	0.72±0.21	0.75±0.13
HF nu	0.91±0.04	0.88±0.06	0.79±0.16	0.79±0.14
LF/HF	0.88±0.07	0.71±0.14	0.64±0.16	0.64±0.10

SD = time domain index of heart rate variability (see text); LF, HF = power in the low- and high-frequency bands, respectively (see text); LF nu, HF nu = normalised units of the low- and high-frequency components (see text).

they reported that CV of HRV indices (6%-15%) were comparable to those observed in our population in similar setting.

Bigger *et al.* (1991) observed less day-to-day variations in the HRV indices in Holter recordings but in very selected populations of coronary artery disease patients. Also Kleiger *et al.* (1987) found that the intraclass correlation coefficient exheeded 0.9 but only in specific time domain indices of HRV (i.e. pNN50 and rMSSD) analyzed from 24-hour samples in normal subjects.

In summary our data, in agreement with other studies, indicate that the reproducibility of HRV parameters is not high: a large day-to-day variations should be considered when HRV determinations are used to assess changes in the autonomic control of the heart particularly during dynamic conditions.

Chapter IV

Comparison of Different Methods for Assessing Sympatho-Vagal Balance in Chronic Heart Failure

A. Abstract

Objectives. To evaluate the ability of different methods to characterise autonomic tone in chronic congestive heart failure we studied 25 patients (aged 62±2 years) with stable moderate to severe ischaemic CHF (New York Heart Association II/III:15/10, ejection fraction: 21.6±2%, peak oxygen uptake: 13.6±0.7 ml/kg/min).

Methods. Sympatho-vagal balance was assessed by 1) Heart rate variability in the time domain assessed by the standard deviation of R-R intervals, 2) Heart rate variability in the frequency domain assessed by low- (LF, 0.03-0.14 Hz) and high-frequency (HF, 0.18-0.40 Hz) components of HRV by autoregressive power spectral analysis, 3) Twenty-four hour, daytime and nighttime heart rate, 4) Submaximal heart rate during upright bicycle exercise, with respiratory gas analysis to obtain peak oxygen uptake and 5) Radiolabeled noradrenaline (NA) spillover.

Results. These methods did not correlate significantly with each other with the exception of day and nighttime heart rate ($r=0.74$, $p<0.001$) and the expected inverse correlation between LF and HF components of HRV ($r=-0.92$, $p<0.001$). No method correlated significantly with peak oxygen uptake, exercise tolerance or ejection fraction.

After 8 weeks of physical training in these CHF patients all methods showed an improvement in autonomic balance: an increase in standard deviation of R-R intervals (+21%, $p<0.02$) and HF component (+41%, $p<0.007$), and a decrease in LF component (-19%, $p<0.002$), LF/HF ratio (-48%, $p<0.03$), NA spillover (-28.9%, $p<0.03$), 24-hour heart rate (-2.7%, $p<0.005$) and submaximal heart rate (-10.8%, $p<0.01$). However, neither the absolute values nor the percentage changes of the individual measures of autonomic function after training, as before, showed significant correlations between each other.

Conclusions. In CHF patients the individual parameters of autonomic control reflect different aspects of circulatory control. A comprehensive description of autonomic tone probably requires multiple methods.

B. Introduction

Cardiac failure is associated with abnormal autonomic control of the cardiovascular system, such as decreased HRV and excessive release of endogenous vasoconstrictor neurohormones, both predictors of increased mortality after myocardial infarction and/or in congestive heart failure (CHF) (Cohn *et al.*, 1984; Kleiger *et al.*, 1987; Bigger *et al.*, 1988). The methods currently used to assess autonomic function do not necessarily measure sympathetic and parasympathetic tone directly. For example (Hasking *et al.*, 1986; Leimbach *et al.*, 1986) NA levels reflect the net effects of both central and peripheral influences on the sympathetic nervous system and are influenced by neuronal uptake and metabolic degradation rates. Heart rate variability, assessed by the standard deviation of the R-R intervals, although primarily reflecting vagal tone is also influenced by the fluctuations in sympathetic tone (Kleiger *et al.*, 1987; Malpas *et al.*, 1990). Power spectral analysis-derived low-frequency fluctuations of HRV are considered a semi-quantitative measure of sympathetic activity and are probably mediated jointly by both the sympathetic and vagal system (Pomeranz *et al.*, 1985). Various methods of estimating vagal activity have not shown a high degree of correlation (Bigger *et al.*, 1989) nor have muscle sympathetic activity or plasma catecholamines shown a consistent correlation with low-frequency heart rate fluctuations (Saul *et al.*,1988 and 1990). We sought, therefore, to further investigate the degree of correlation between multiple individual measures of autonomic function in a homogeneous group with impaired autonomic control: patients with CHF in whom autonomic balance is both abnormal and prognostically important. Such information may guide whether individual measures suffice to describe autonomic control in these patients or whether multiple independent methods are necessary. We compared the changes in the various parameters of autonomic function before and after physical training, which has been reported (Coats *et al.*, 1992a) to alter autonomic balance.

C. Methods

i. Study Population

Twenty five subjects gave informed consent for this trial, which was approved by the local ethics committee. We studied only patients with stable CHF secondary to ischaemic heart disease; the ischaemic aetiology was evidenced by documented previous myocardial infarction and/or coronary arteriography and coronary artery bypass surgery. However there was an absence of symptomatic angina or electrocardiographic evidence of ischaemia limiting exercise; there was also an absence of Holter monitoring evidence of ventricular arrhythmias. Patients were characterised by limitation of exercise by symptoms of dyspnoea or fatigue only and were able to reach a respiratory exchange ratio of at least unity.

Patients were aged 62±2 (range 43-75 years). Fifteen were New York Heart Association class II and ten class III. Radionuclide left ventricular ejection fraction was 21.6±2% and peak oxygen uptake 13.6±0.7 ml/kg/min (mean±SEM). Nine had undergone coronary artery bypass grafting. All subjects were taking diuretics (median frusemide dose 80 mg); twenty two out of 25 were on ACE inhibitors. Pharmacological treatment was stable for 3 months prior to study and for the duration of the study in all subjects. Upon entry to the trial, patients underwent a 2-4week familiarisation and baseline evaluation phase during which reproducible exercise and autonomic tests were obtained. Subsequently all patients were randomised to 8 weeks bicycle exercise or avoidance of exercise in a crossover design, that has been previously described (Coats *et al.*, 1990).

ii. Exercise Testing

Exercise tests were performed on a Tunturi Professional Ergometer (Tunturi, Finland). The upright bicycle tests were performed in 5-min stages, with 25-Watt increments to the limit of tolerance. All tests were performed before daily medication had been taken and were conducted by a neutral "blinded" observer. Oxygen consumption and CO_2 production were measured during the test (Coats *et al.*, 1990).

iii. Heart Rate Measurements

1. *Heart rate during the exercise tests.* Heart rate was recorded from the electrocardiogram during each stage of the bicycle exercise test.
2. *Twenty four-hour ECG monitoring.* Twenty four-hour electrocardiographic monitoring was performed using a 2-channel recorder (Oxford Medilog II, Oxford Med. Instruments). Modified V_1 and V_5 leads were recorded. Two channel quantitative electrocardiogram analysis was performed using a computerised non-triggered template system consisted of a Z80A preprocessor and DEC-LSI master, developed and validated in our laboratory (Rossi *et al.*, 1983). Analysis of the recordings was performed blind to other patient characteristics. The data were analysed to obtain the mean value and the standard deviation of all R-R intervals, which had normal morphology and cycle lengths within 80 and 120% of the preceding cycle duration. Mean heart rate and HRV (SD of R-R intervals) are presented for the whole 24 hours, daytime (14-20 hours) and night (0-6 hours).
3. *Laboratory rest heart rate monitoring.* Six hundred and forty consecutive heart beats were recorded in a quiet darkened enviroment after 30 minutes bed rest during the day on a Store 4 Racal-Thermionic FM tape recorder (Southampton, UK), at 15/16 inches/sec.

iv. Power Spectral Analysis of the R-R Variability

1. *Signal acquisition.* The tape recorded data were digitized off-line by a 12 bit analog-to-digital converter (NB-MIO-16 board, National Instruments, Austin, Tx) at a sampling rate of 500 samples/sec. The converter was connected to a MacIntosh IIcx computer (Apple Inc., Coupertino, Ca) equipped with 5Mb RAM memory and a 60Mb hard disk. A "C" language programme identified all the QRS complexes in each sequence and then located the peak of

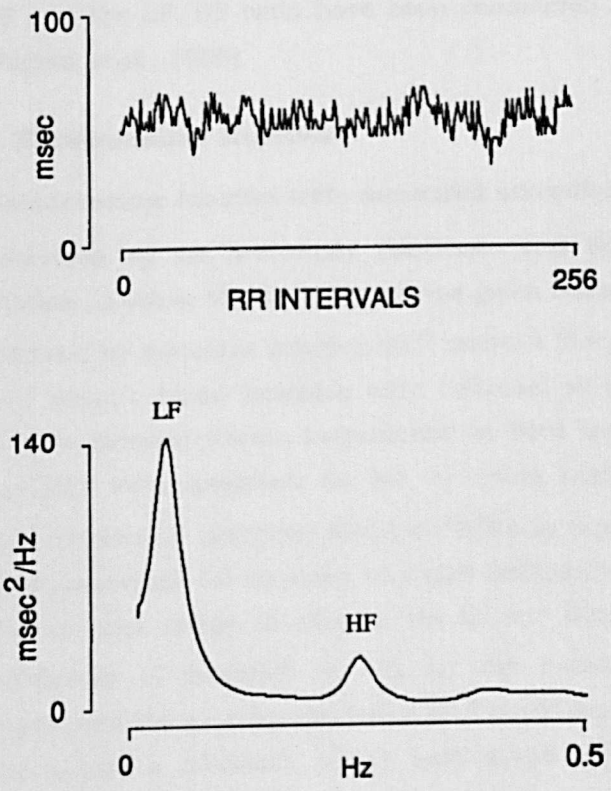

Figure 4.1. Upper panel showing the histogram of R-R interval variability for one of the subjects; lower panel showing power spectral analysis of this R-R variability with two separate peaks: the high-frequency peak (0.18-0.40Hz, HF) which is synchronous with respiration and the low-frequency peak (0.03-0.14 Hz, LF) which has been found to reflect changes in sympathetic tone.

each R wave. From these data, the R-R intervals were obtained and for each sequence, 256 heart beats were analyzed. Trends were removed from each sequence by subtraction of that same sequence after a 124-lag window smoothing procedure, following a previously described algorithm (Kay and Marple, 1981). Premature beats were interactively identified and corrected by linear interpolation with the previous and following beats.

2. *Power spectral analysis.* Power spectral analysis of the R-R interval signal was performed by an autoregressive model (Kay and Marple, 1981). Model coefficients were evaluated according to a modification of the Burg algorithm (Ulrych and Bishop, 1975). Model order was assessed by Akaike criteria (Pagani *et al.*, 1986) and a model order between 9 and 13 was found to be adequate in most cases. Spectral components were obtained by a decomposition method previously described (Isaksson *et al.*, 1981; Kay and Marple, 1981; Pomeranz *et al.*, 1985), which was also used to measure the area below each spectral peak. We expressed the variation in Hz rather than in cycles/beat, assuming that the R-R interval changes were small with respect to the mean R-R interval and considering the sampling time equal to the mean R-R interval. An example of this spectral analysis for 1 subject is shown in Figure 4.1. The power spectrum shows two separate peaks: the higher frequency peak is related to respiration; the low-frequency peak appears unrelated to any respiratory event and has been found to reflect changes in sympathetic tone (Pomeranz *et al.*, 1985; Pagani *et al.*, 1986). We evaluated the power of the harmonic components in the region between 0.03 and 0.14Hz (LF component) and those in the range between 0.18 and 0.40Hz (HF component). In order to simplify comparison between spectra obtained in different units, we considered the relative percentage of each spectral component compared to the total oscillatory power [normalised units (nu)].

The relative amount of HF, and the absolute amount of respiratory sinus arrhythmia, have been considered as indices of parasympathetic activity, whereas the relative amount of

LF and the LF/HF ratio have been considered primarily as indices of sympathetic activity (Pagani *et al.*, 1986).

v. Noradrenaline Kinetics

Noradrenaline kinetics were measured according to the techniques of Esler *et al.* (1979) and described by us previously (McCance and Forfar, 1990). 1-[2,5,6-^{3}H]NA (New England Nuclear, Boston Massachusetts) was given intravenously as a bolus [12 microCi (0.44 MBq)] followed by constant infusion [0.7 microCi (0.026 MBq)/min/m^2] for up to 60 min. Arterial and venous blood samples were collected at rest and at submaximal (50 Watts) supine bicycle exercise (5-min increments) in both training and detraining conditions. The blood samples were analysed for NA by using high performance liquid chromatography with electrochemical detection and for [^{3}H]NA by liquid scintillation counting of alumina extracts with correction for recovery of a non-radioactive internal standard (dihydroxybenzylamine). The normal range of plasma NA in our laboratory is 120-300 pg/ml. The within day coefficient of variation is 4% for the estimation of plasma NA and 7% for [^{3}H]NA. Noradrenaline plasma clearance and whole-body NA spillover were measured according to the following relations, which hold under steady state conditions (McCance and Forfar, 1989a)

$$\text{Whole body NA clearance} = \frac{\text{Infusion rate (dpm/min)}}{\text{Plasma [}^3\text{H]NA (dpm/ml)}}$$

$$\text{Whole body NA spillover} = \frac{\text{Infusion rate (dpm/min)}}{\text{Specific radioactivity of plasma NA (dpm/pg)}}$$

(dpm is disintegrations per minute of tritiated NA).

It has been shown that after the attainment of steady-state conditions at rest, the plasma concentration of endogenous and tritiated NA are in a new steady-state condition after 5 min of exercise (Hasking *et al.*, 1986).

vi. Statistical Analysis

Statistical analysis was carried out according to the recommendations of Hills and Armitage for crossover trials (1979). Student's paired t-test was used for comparisons in heart rate and the Wilcoxon signed-rank test was used for comparisons in HRV, in LF and HF components of power spectral analysis and in NA spillover between physical training and detraining. Least squares linear regression analysis was also carried out between variables

with the "Statview" 512 statistical programme for the Macintosh computer. For intermethod correlations, a 1% level of confidence or lower was considered significant, taking into account the multiple possible regressions. Results are expressed as mean±SEM.

D. Results

i. Intermethod Comparisons

Individual patient demographic and autonomic data are shown in Table 4.1; examples of

TABLE 4.1. Individual Patient Demographic and Autonomic Data

ID	Drugs	EF (%)	VO_2max (ml/kg/min)	Ex-Dur (mins)	HRa (bpm)	HRs (bpm)	Smax-HR (bpm)	SD-RRa (msec)	SD-RRs (msec)	PSA-LF (nu)	PSA-HF (nu)	LF/HF	NA-50W (ng/min/m^2)	NA-50W (ng/min/m^2)
1	D; I	35	15.8	17.6	62	65	90	47.7	67.7	78.5	15	5.2	189	238
2	D; Dig	15	19.9	17	100	78	126	16.9	52	34.6	56	0.6	529	
3	D; I	12	15.4	14.5	72	77	123	32.1	72.1	72.6	16.4	4.4	512	987
4	D; I		9.5	9	77	65	102	29.9	58	91.4	5.3	17.2	213	399
5	D; I	14	11.1	7	87	87	115	15.3	18.3	76.4	16.5	4.6	490	804
6	D; I	19	12	13	98	80	113	35.1	51.1	87.7	9.5	9.2	491	907
7	D; I	28	11.8	11	60	81	110	108.4	122.9	72.9	17	4.3	432	617
8	D; I	18	14.7	18	77	73	123	33.9	43.4	95.2	4.8	19.8	328	769
9	D; I	24	20.3	20	56	58	106	63.3	71.8	23.7	71.3	0.3	372	350
10	D; I	34	16.4	20	49	52	98	26.5	32	41.4	58.5	0.7	330	726
11	D; I	16	6.5	8	91	82	118	17.6	23.1	69	28.3	2.4	555	839
12	D; I	39	16.3	14	64	60	110	40	44.5	52.3	43.9	1.2	1285	1525
13	D; I	34	10.8	9	72	75	96	32.5	43	67.6	29.4	2.3	526	743
14	D; I	15	12.7	9.5	60	62	92	18.4	27.9	69.2	23.9	2.9	529	1567
15	D; I	22	16.5	16	84	74	104	21.5	99.8	81.2	11.2	7.3	209	533
16	D; I	24	16.2	12	68	73	102	18.6	24.9	76.2	17.2	4.4	308	563
17	D; I	16	11.7	16	75	68	85		155.2				342	586
18	D; I	9	13.7	13	64	61	97	11.4	102.5	60.7	12.4	4.9		
19	D; I	25	11.4	14	62	68	99	57.2	90.3	29.1	70.8	0.4		
20	D; I	15	9.7	9	91	94	114	17.8	63.3	71.1	24.1	3		
21	D; I; A	39	12.9	18.6	69	67	81	36.3	46.8	40.4	45.5	0.9		
22	D; Dig	11	12.2	12.5	75	66	90	13.2	73.4	46	30.4	1.5		
23	D; A	18	18.9	18	72	69	94	30.7	78.1	43.3	31.9	1.4		
24	D; I	18		18.3	75	75	100	41.7	65.6	61.1	38.8	1.6		
25	D; I	19	8.7	9	77		101							

D = Diuretics; I = Angiotensin converting inhibitors; Dig = Digoxin; A = Antiarrhythmics; EF = Ejection fraction; VO_2max = Peak oxygen uptake; Ex-Dur = Upright bicycle exercise duration; HR = Heart rate; a = Awake (resting laboratory recording of 640 consecutive beats); s = sleep (0-6am); Smax-HR = Submaximal heart rate during upright bicycle exercise; bpm = Beats per minute; SD-RR = standard deviation of R-R intervals; PSA = power spectral analysis of heart rate variability; LF = low-frequency (0.03-0.14Hz) and HF = high-frequency (0.18-0.40Hz) components of heart rate variability; NA-rest and NA-50W = Noradrenaline spillover at rest and during submaximal (50 Watts) supine bicycle exercise.

some of the possible intermethod correlations are shown in Figure 4.2. Only 7 out of the 45 possible correlations were significant at the 1% level. These correlations were all between

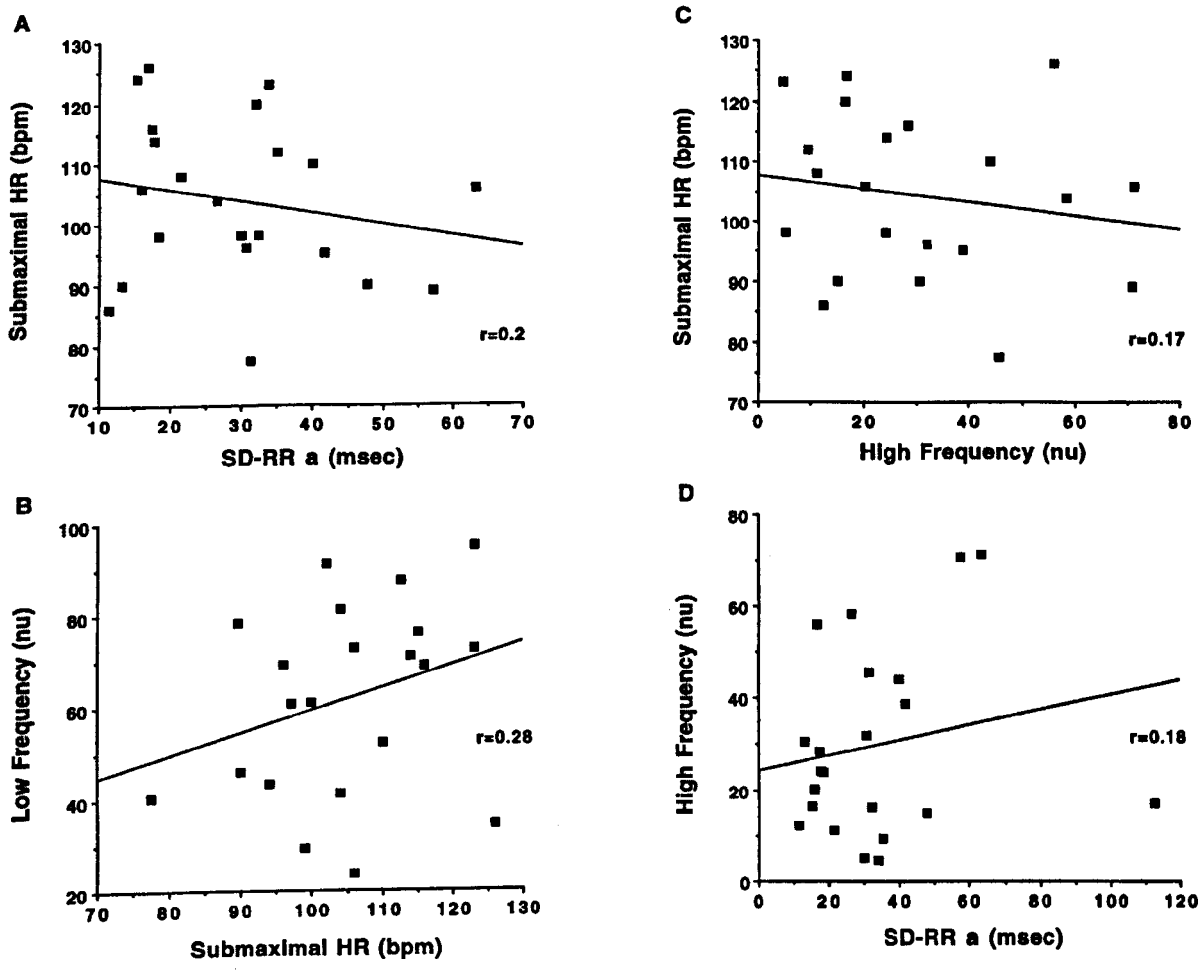

Figure 4.2. A) Correlation between submaximal heart rate (HR) and standard deviation of R-R intervals (SD-RR) during awake (a) resting laboratory recording; B) Correlation between low-frequency component of heart rate variability and submaximal HR (nu = normalised units); C) Correlation between high-frequency component of heart rate variability and submaximal HR (nu = normalised units); D) Correlation between high-frequency component of heart rate variability and standard deviation of R-R intervals (SD-RR) during awake resting laboratory recording.

closely similar techniques (such as daytime and nighttime heart rate), whereas between differing techniques (such HRV and NA spillover) there was no correlation (correlation matrix in Figure 4.3). Thus, there was a good correlation between heart rate awake and heart rate during sleep ($r=0.74$, $p<0.001$) and between either heart rate awake or heart rate sleep and submaximal heart rate ($r=0.51$ and $r=0.54$ respectively, $p<0.01$). Also, as expected, there were good correlations between LF and HF ($r=-0.92$, $p<0.001$), LF and LF/HF ratio ($r=0.77$, $p<0.001$) and between HF and LF/HF ratio ($r=-0.69$, $p<0.001$). Finally there was good correlation between NA spillover at rest and at submaximal exercise ($r=0.77$, $p<0.001$) (Figure 4.3). There was no significant correlation, however, between the autonomic parameters and indices of cardiac function (ejection fraction, peak oxygen uptake or exercise duration).

The effects of physical training on the individual measurements of autonomic balance are shown in Table 4.2.

TABLE 4.2. Values of the Various Parameters Measuring Autonomic Balance; Effects of Physical Training

Parameter	**Value**	**% Change**	**p-Value**
24-hour heart rate (bpm)	76.0±2.9	-2.7	<0.005
Heart rate sleep (0-6 am)	71.3±2.0	-2.8	<0.030
Heart rate awake	73.4±2.6	-2.8	<0.05
Heart rate day-time (14-20 pm)	86.7±3.3	-3.4	<0.05
Submaximal heart rate	103.6±2.4	-10.8	<0.01
SD of R-R intervals, sleep (msec)	63.7±6.8	+20.7	<0.02
SD of R-R intervals, awake (msec)	33.3±4.5	+20.8	<0.004
Low-Frequency (nu)	62.7±4.2	-18.9	<0.002
High-Frequency (nu)	29.5±4.2	+40.5	<0.007
Low/High-Frequency ratio	4.4±1.1	-47.7	<0.03
Noradrenaline, rest (ng/min/m^2)	449±60.0	-28.9	<0.03
Noradrenaline, 50 Watts	760±92.0	-9.3	NS

Again there was no correlation between the individual parameters after training or between the training-induced percentage (%) changes of the various parameters of autonomic function apart from the self-evident ones between LF and HF ($r=-0.61$, $p<0.003$), LF and LF/HF ratio ($r=0.64$, $p<0.001$) and between HF and LF/HF ratio ($r=-0.73$, $p<0.001$) (correlation matrix in Figure 4.4).

There were only 2 patients taking digoxin and only 3 not taking an ACE inhibitor so that these numbers were too small to determine whether these medications significantly affected the lack of correlation between the various parameters of autonomic control.

The possibility that prior coronary artery bypass surgery may have altered both afferent and efferent cardiac neural mechanisms via structural injury cannot be excluded. However, when we excluded patients (9 out of the 25) who underwent coronary artery bypass surgery from analysis, the lack of correlation between different methods of assessing sympatho-vagal balance still exists.

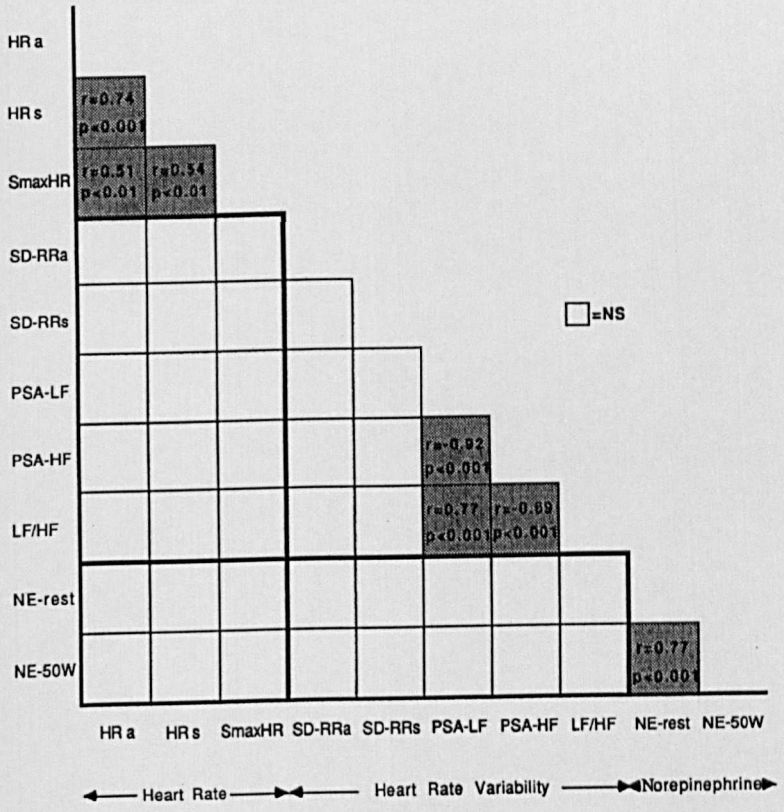

Figure 4.3. Comparisons between the various parameters of autonomic function at baseline. NS = not significant; other abbreviations as in Table 4.1.

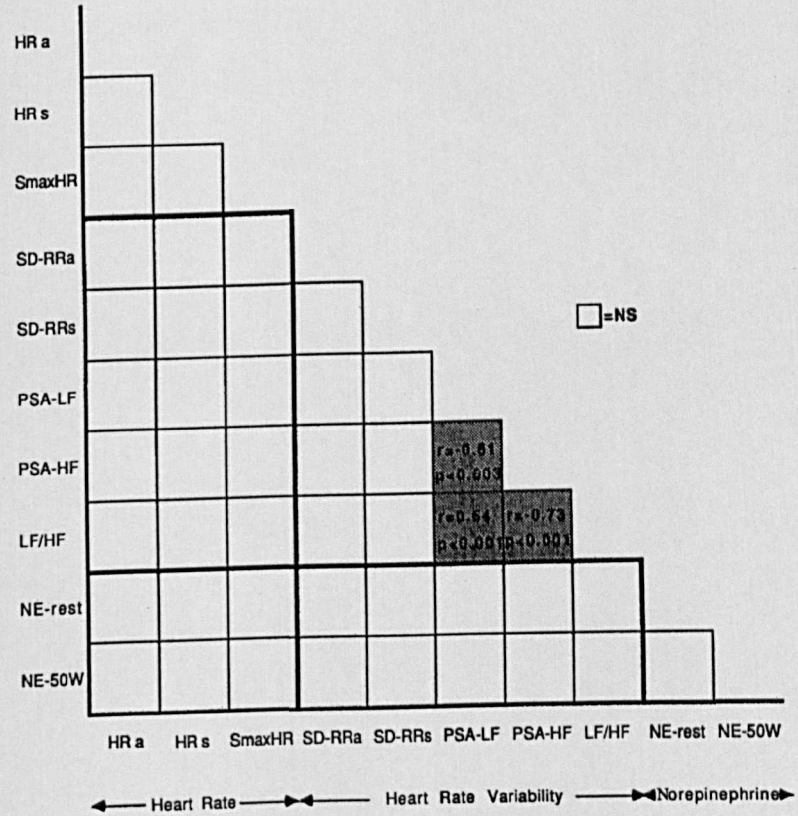

Figure 4.4. Comparisons between the percentage (%) changes of the various parameters of autonomic function after physical training. NS = not significant; other abbreviations as in Table 4.1.

E. Discussion

Methodological considerations seem to be of particular importance in relation to studies of the autonomic changes in CHF. The methods currently used to evaluate autonomic balance in patients with CHF should be comparable if only a single descriptive measure is to be used. Venous plasma NA is clearly not an ideal guide to sympathetic activity in heart failure (Esler *et al.*, 1988), because the level of plasma NA can be altered by changes in both neural release and in removal from plasma, and CHF is a state in which clearance is impaired (McCance and Forfar, 1990). Another difficulty is that circulating levels are the results of spillover of NA into the circulation from the whole-body but cardiovascular stimuli elicit highly differentiated responses that seldom involve a simple overall increase or decrease in sympathetic neural activity (McCance and Forfar, 1989b). Elevated levels of circulating NA may result either from increased sympathetic nerve activity, from facilitated release of NA from peripheral adrenergic nerve endings or from altered synthesis or metabolism of NA. For example, angiotensin II stimulates the synthesis of NA in nerve terminals and facilitates NA release (Hughes and Roth, 1971).

A reasonable correlation has been described between resting muscle sympathetic nerve activity and plasma NA levels in patients with CHF (Leimbach *et al.*, 1986). In this study, however, the range of the severity of heart failure was broad, the age distribution wide, the aetiologies of heart failure multiple and the number of subjects was small, all of which could increase the apparent correlation between methods. Recent data have shown different time courses of both acute changes and their magnitude, when comparing plasma NA levels and peroneal nerve muscle sympathetic activity (Rea *et al.*, 1990).

Although the LF component derived from power spectral analysis of the R-R interval variability has been said to be a quantitative measure of sympathetic tone (Pomeranz *et al.*, 1985; Pagani *et al.*, 1986), the contribution of vagal activity and baroreflex responses in the genesis of LF fluctuations is not trivial. This may explain the lack of correlation between LF or LF/HF ratio and NA spillover in our study. Our findings are in keeping with those of Saul *et al.* (1990) in normals where muscle sympathetic activity did or did not correlate with LF heart rate fluctuations depending on the vagal influence to the sinus node. These investigators also showed no correlation between plasma NA levels and the absolute or relative power of the LF spectral component in CHF patients (Saul *et al.*, 1988).

ß-adrenoreceptor down-regulation, frequently observed in CHF (Bristow *et al.*, 1982), might also lead to an effective de-coupling of sympathetic nerve activity (as reflected by NA spillover) from end-organ response (as reflected by measures of HRV). Variability in the extent of ß-adrenoreceptor down-regulation and responsiveness between subjects would need to be taken into account.

We also did not find a significant correlation between the HF component derived from power spectral analysis of R-R variability and the standard deviation of R-R intervals (over 24 hours or during daytime or nighttime). This finding is compatible with the study of Bigger *et al.* (1989), which shows very high correlation only among electrocardiographic

measures of high-frequency HRV but very weak between these measures and the standard deviation of R-R intervals, the only parameter that has been shown to predict sudden cardiac death after a myocardial infarction (Kleiger *et al.*, 1987). The standard deviation of R-R intervals, may incorporate an effect of sympathetic activity, which drives low-frequency heart rate variations. In CHF such variations may constitute a higher proportion of HRV. The very good correlation, found by Hayano *et al.* (1991), between the time and frequency domain indices of HRV was assessed after eliminating sympathetic effects on the heart rate by ß-blockade. Furthemore these findings were described in normal subjects with a wide range of autonomic function, whereas heart failure is a condition with severe impairment in sympatho-vagal interactions and a narrower range of autonomic function.

In the interpretation of the data the confounding influence of the ACE inhibitors and digitalis should be taken into account. Almost all of the patients were on ACE inhibitors, which have been suggested to increase vagal activity in CHF (Osterziel *et al.*, 1988). However, to our knowledge there are no additional studies to have examined the intermethod correlations in patients off medication, due mainly to the importance of ACE inhibition in the management of moderate to severe CHF. Digitalis has been demonstrated to produce early, profound and sustained reductions in sympathetic nerve activity in patients with CHF (Ferguson *et al.*, 1989), but there were only 2 subjects taking this agent in this study rendering the number too small to determine the significance of digitalis in the correlations between the autonomic parameters.

In this study whole-body NA spillover, power spectral analysis components of R-R interval variability and the standard deviation of R-R intervals failed to predict left ventricular ejection fraction, peak oxygen uptake and exercise tolerance. This is in agreement with previous studies (Viquerat *et al.*, 1985; Leimbach *et al.*,1986) and suggests that autonomic dysfunction alone may not be the primary limiting factor to exercise in these patients. Skeletal muscle structural and functional abnormalities (Mancini *et al.*, 1989 and 1992b; Duscha *et al.*, 1999; Hambrecht *et al.*, 1999) as well as endothelial dysfunction (Ferrari *et al.*, 1998a; Hambrecht *et al.*, 1998), associated with CHF, could also contribute significantly to exertional fatigue, characterising this category of patients.

Despite the improvement in all individual measures of autonomic control after training, reflecting beneficial changes in the autonomic balance and/or baroreflex-cardiopulmonary receptor activity, there was again no correlation between the training-induced changes in those parameters (Figure 4.4), indicating the difficulty in obtaining precise measures of autonomic function. Future studies should also control for conditioning status when assessing autonomic function in heart failure either in cross-sectional or intervention studies. The lack of inter-method correlation leads to the conclusion that in heart failure individual measures of autonomic balance represent different aspects of circulatory control. Therefore a comprehensive description of the autonomic status may necessitate a panel of complementary methods.

i. Limitations of the Study

The ischaemic aetiology of the heart failure in our group of patients does not permit us to extrapolate the findings of this study in patients with dilated cardiomyopathy or CHF secondary to either hypertension or valvular disease.

Although, by excluding those patients who underwent coronary artery bypass surgery from analysis, the lack of correlation between different individual autonomic indices still exists, the possibility that prior coronary artery surgery may have structurally damaged both afferent and efferent cardiac neural mechanisms still remains.

Almost all of the patients were on ACE inhibitors, which have been shown to increase vagal tone in CHF. Therefore, our results pertain only to patients taking ACE inhibitors. In contrast, there were only 2 subjects taking digitalis, too small to determine if digoxin might have affected our conclusions.

Chapter V

Effects of Physical Training on Skeletal Muscle Metabolism in Experimental Heart Failure

A. Abstract

Objectives. Recent investigations have established the presence of intrinsic skeletal muscle metabolic abnormalities in CHF. The influence of physical training on skeletal muscle metabolism after myocardial infarction was studied in a rat model of the development of heart failure.

Methods. Phosphorus-31 magnetic resonance spectroscopy (MRS) and enzyme assays were performed in female Wistar rats 12 weeks after coronary artery ligation and in a non-trained sham-operated control group. Infarcted rats were allocated randomly to either 6 weeks of training or non-training. Phosphorus-31 spectra were collected from the calf muscles during sciatic nerve stimulation at 2 Hz maximally and at 200g twitch tension. From the spectra the areas and peaks of phosphocreatine (PCr) and inorganic phosphate (Pi) were measured and pH was derived from the chemical shift between these two peaks. Fibre typing and enzymatic assays were performed on the calf muscles of the contralateral non-stimulated leg to measure the mitochondrial-cytoplasmic enzyme glutamate pyruvate transferase, the glycogenolytic enzyme phosphorylase and the mitochondrial oxidative enzymes citrate synthase and beta-hydroxyacyl CoA dehydrogenase.

Post-mortem rats were also divided into congestive heart failure (MI-CHF) and non-congestive but with left ventricular dysfunction (MI-LVD) animals according to the lung/body weight ratio. These results exactly matched a subdivision by myocardial infarct size. Results were analysed by factorial analysis of variance followed where appropriate by individual comparisons with Scheffe's procedure.

Results. At maximum twitch tension, the muscle pH of the trained MI-CHF animals was significantly higher than in the non-trained MI-CHF animals but not different from the pH of the MI-LVD animals (trained or not) or the sham-operated animals. At maximum twitch tension, the PCr/(PCr+Pi) ratio was lower, but not significantly, in the non-trained MI-CHF group than in the sham-operated group or the trained MI-CHF group. At 200 g twitch tension, PCr and pH were found to be significantly lower in the non-trained MI-CHF group compared with all the other groups. Phosphocreatine and adenosine diphosphate (ADP) recovery half-times were significantly longer in the MI-CHF non-trained group compared with all the other groups. The training did not induce a change in the enzymatic activities in the infarcted animals with left ventricular dysfunction but did correct the lower mitochondrial-based oxidative citrate synthase activity in the non-trained MI-CHF animals.

A trend towards a higher glutamate pyruvate transferase activity was found in both MI-CHF and MI-LVD groups after training compared to the non-trained animals. This normalisation of muscle metabolism was seen without any change in calf muscle mass or individual fibre size between trained and non-trained animals, indicating that the training effects are independent of muscle mass and, therefore, making atrophy unlikely to be the sole cause of the metabolic abnormalities in heart failure.

Conclusions. It is concluded that training in rats with congestive heart failure can reverse the abnormalities of skeletal muscle metabolism found by MRS and by measurement of the mitochondrial-based enzyme citrate synthase, indicating a contribution of decreased physical activity in the aetiology of these changes.

B. Introduction

Recent investigations suggest that intrinsic skeletal muscle abnormalities could explain exertional fatigue in patients with CHF (Wilson *et al.*, 1993a). Abnormal ^{31}P MRS responses to forearm or calf muscle exercise have been demonstrated in patients with CHF (Wilson *et al.*, 1985c; Wiener *et al.*, 1986; Arnolda *et al.*, 1990; Marie *et al.*, 1990). The aetiology of these muscle metabolic changes is unclear but reductions in mitochondrial enzymes have been noted, suggesting an oxidative defect (Arnolda *et al.*, 1991). A reduction in blood flow to skeletal muscle does not appear to be the only cause (Wiener *et al.*, 1986; Massie *et al.*, 1987a) because changes have been observed even during ischaemic exercise (Massie *et al.*, 1988), but whether chronically inadequate blood flow is involved in the genesis of these changes is not known. Contrary to heart failure, an inadequate blood flow without heart failure (peripheral vascular disease) induces an increased level of enzyme activities (Holm *et al.*, 1972 a&b; Bylund *et al.*, 1976; Elander *et al.*, 1985) making it unlikely that chronically impaired nutritive muscle blood flow is sufficient alone for the development of the observed changes. Another feature of the syndrome of CHF is prolonged muscular inactivity and it is not clear to what extent deconditioning in skeletal muscles contributes to the abnormal metabolism in CHF. It has been recently shown that muscle atrophy, which is sometimes significant in patients with CHF, can only very modestly contribute to the skeletal muscle metabolic abnormalities and exercise intolerance seen in CHF (Mancini *et al.*, 1992b). Several studies have shown that physical training of patients is beneficial in improving exercise tolerance, ventilation, autonomic function and symptoms in subjects with moderate or severe left ventricular dysfunction (Sullivan *et al.*, 1988a; Coats *et al.*, 1990 and 1992a). In our study we sought to investigate the relative importance of left ventricular dysfunction and physical training on skeletal muscle metabolic abnormalities and histology in experimental heart failure. Muscle metabolism of the hindlimb was studied by ^{31}P MRS before, during and after sciatic nerve stimulation, and by enzyme activity measurements in five groups of animals: sham-operated, trained MI-CHF heart failure, trained MI-LVD, non-trained MI-CHF and non-trained MI-LVD animals.

C. Methods

i. Coronary Artery Ligation

The left coronary artery was ligated by a modified method described by Pfeffer *et al.* (1979) in female Wistar rats weighing 160-180 g. The rat was anesthetised with ether, intubated and ventilated. Anaesthesia was maintained with Halothane (1%) in a mixture of N_2O/O_2. A thoracotomy was performed and a ligature was placed around the left coronary artery. In sham-operated animals this ligature was left untied. Operative mortality was about 60% within 24 hours of myocardial infarction. All sham-operated animals survived.

ii. Training

Six weeks after the operation, the surviving infarcted rats were allocated randomly to either 6 weeks training or non-training. In the trained group, the rats were placed on a treadmil at a speed of 15m/min and the exercise was performed for 30 min/day, 6 days per week.

iii. Magnetic Resonance Spectroscopy Experiments

At the end of the training or non-training period, anaesthesia was induced with pentobarbitone sodium (30mg/kg) and was maintained during each experiment with halothane (0.5-1% in 1:1 N_2O/O_2) delivered through a face-mask. Sciatic nerve stimulation at 2 Hz was used to cause contraction of the plantaris, gastrocnemius and soleus muscle as a group. The preparation of the rats has been previously described (Shoubridge *et al.*, 1984). The left leg was immobilised by pinning the ankle and knee joints. The distal tendon of the flexor muscles complex was attached to a force transducer. Twitch tension was measured as the difference between peak of active and resting muscle.

All experiments were performed at a frequency of 73.8 MHz for ^{31}P in a vertical wide bore 4.3 T magnet. The radiofrequency coil was a Dadok coil surrounding the plantaris-gastrocnemius-soleus group of muscles of the rat leg. The same coil was used for both transmission and reception. Free induction decays (FIDs) were collected as 2K data with a sweep width of 2000 Hz. The study was done using a tip angle of 60° and 2-sec interpulse delay.

A first acquisition of 32 pulses (80 sec) was obtained at rest. Then the sciatic nerve was stimulated for 640 sec at the maximum obtainable twitch tension. This permitted aquisition of 8x32 pulse spectra from the muscle during exercise and then a 640 sec recovery was allowed during which a further 4x8 pulse spectra and then 7x32 pulse spectra were collected. The 32-pulse spectrum acquired at the end of the recovery period was used to assess the completeness of the recovery of the muscle. The sciatic nerve was again stimulated during 320 sec with a calibrated submaximal sciatic nerve stimulation allowing a twitch tension of 200 g in order to normalise the twitch tension in all the animals studied. We used this additional stimulation protocol in our animals to avoid the variation in tension

produced in the maximal stimulation protocol resulting from changes in local collection of fluid around the nerve and the electrodes.

The broad component underlying the PCr and Pi region was reduced by multiplying the first 4 data points by a trapezoidal function. An exponential line broadening of 15 Hz was then applied. After a Fast Fourier Transformation, the peak areas were calculated using a Lorentzian line fitting program (Glinfit-Bruker Spectrospin). The pH was calculated from the chemical shift of Pi from PCr using the usual formula (Moon and Richards, 1973). Skeletal muscle [ATP] does not alter in infarcted rats so the absolute concentrations of Pi and PCr could be calculated from the ratios of the areas under the peaks of Pi, PCr and ATP.

From the spectra obtained at rest, during stimulation of the muscle at maximum twitch tension or 200 g twitch tension and during subsequent recovery, several parameters were studied: pH, PCr/(PCr +Pi) and the maximum tension reached during the stimulation and the recovery half-times for PCr and ADP. Recovery half-times for PCr and ADP after maximal twich tension were calculated by graphical interpolation.

iv. Skeletal Muscle Enzyme Activity

At the end of the MRS experiments all the calf muscles of the non-stimulated contralateral leg were taken and freeze clamped. Enzyme assays were performed on the contralateral non-stimulated leg in the animals awaiting assay for two mitochondrial oxidative enzymes citrate synthase and beta-hydroxyacyl CoA dehydrogenase (an enzyme reflective of fat oxidation), the glycogenolytic enzyme phosphorylase and the cytoplasmic-mitochondrial enzyme glutamate pyruvate transferase, which has proved to be increased by physical training and which favours the removal of pyruvate through the alternative pathway of alanine, thus avoiding the excessive formation of lactate. Ground frozen tissue was extracted in 0.1 mM Imidazole, 1mM EGTA, 5mM $MgCl_2$ at pH 9.2 and assayed for citrate synthase activity by the method of Srere (1969). Beta-hydroxyacyl CoA dehydrogenase was measured as described by Bass *et al.* (1969). Total phosphorylase activity in the presence of 3mM 5'-AMP was measured using 3MM filter paper by the method of Gilboe *et al.* (1972). Glutamate pyruvate transferase activity was measured according to the method described by Mole *et al.* (1973). Specific enzyme activities were expressed as $\mu mol.min^{-1}$ per gram of wet weight at 30° C.

v. Determination of Infarct Size, Right Ventricular and Lung Wet Weight

At the end of the experiment, the chest was opened and the heart and the lungs were removed. The free wall of the right ventricle (RV) was dissected from the left ventricle (LV) and the interventricular septum. After weighing the RV, the LV and the lungs, the LV was fixed in formalin for further determination of the infarct size. Four to 6 transverse slices of LV were cut from apex to base. Micrometer sections were cut and stained with Massons' trichrome stain. With a planimeter image analyser, epicardial and endocardial circumferences were measured and infarct size was expressed as percentage of the summed circumference of the LV (Drexler *et al.*, 1985). Infarct size, RV weight and lung weight have

been extensively shown to be correlated to the haemodynamic data in infarcted rats and were consequently used to assess heart failure in these animals (Pfeffer *et al.*, 1979; Bech *et al.*, 1989).

vi. Calf Muscle Weight

Calf muscle mass was measured in each group. The limb of the stimulated leg was transected at the knee and at the tibiotarsal joint, the skin was removed and the muscle and connective tissue were stripped from the lower leg and weighed carefully. The calf muscle wet weight was expressed in absolute values and as the ratio to body weight.

vii. Calf Muscle Histology

A specimen of gastrocnemius muscle was orientated transversely on filter paper and was snap-frozen in isopentane cooled in liquid nitrogen. The muscle was sectioned on a cryostat (9μm thick) and serial sections were stained using routine histochemical procedures for adenosine triphosphatase (ATPase) at pH 9.5, 4.6 and 4.35/4.2, and for nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) (Dubowitz, 1985b).

The specimens were examined with an optical microscope (Zeiss Axioskop) and photographs from matching serial areas were taken of each of the 4 histochemical stains. The photomicrographs were compared and the characteristics of each matched fibre was determined by reference to their staining pattern. At least 100 muscle fibres were marked and measured for each specimen. Fibre diameter was measuerd by recording the least dimension of the fibre perpendicular to the long axis, in order to correct for the errors inherent in measuring tangentially cut fibres (Dubowitz, 1985a).

"The investigation was performed in accordance with the Home Office *Guidance on the operation of the animals (Scientific Procedures) Act 1986,* published by Her Majesty's Stationery Office (HMSO), London".

viii. Statistical Analysis

In order to analyse the data, the animals were divided in five groups: infarcted animals, trained, with no heart failure (MI-LVD) (group 1), infarcted animals, trained, with congestive heart failure (MI-HF) (group 2), non-trained infarcted animals with MI-LVD (group 3), non-trained infarcted animals with MI-HF (group 4) and sham-operated (i.e. non-infarcted) animals (group 5). Post-mortem animals were assigned to the congestive heart failure group when the wet lung weight/body weight ratio was higher by at least two standard deviations than the mean calculated in the sham-operated animals (Table 5.1). This division also separated the animals with RV weight per body weight greater than 1 mg/g. Because of differences in muscle mass, the sham-operated animals can only be used for comparison of resting and recovery data and enzymatic analysis. As a result, the exercise data have been omitted from Table 5.2. The non-trained groups acted as controls for the appropriate trained group.

Results are expressed as the mean±standard deviation. Two-factorial analysis of variance was used to look at the differences between the various groups of animals, with major factors being the training status and the infarct size. If both factors were significant by ANOVA, individual differences between the 5 groups were further examined by Scheffe's procedure. Only the training status was considered for comparisons between non-trained, trained and sham-operated animals when there was no significant effect of infarct size on the factorial ANOVA. $P<0.05$ was considered as indicating a significant difference. Correlations were determined by Srearman's correlation coefficient.

D. Results

1. Organ Weight

As expected from the selection criteria, the lung wet weight/body weight ratio was higher in the two groups with congestive heart failure (Table 5.1) and was correlated well with the percentage of LV circumference which was infarcted ($r=0.96$, $p<0.001$; Figure 5.1 left panel)

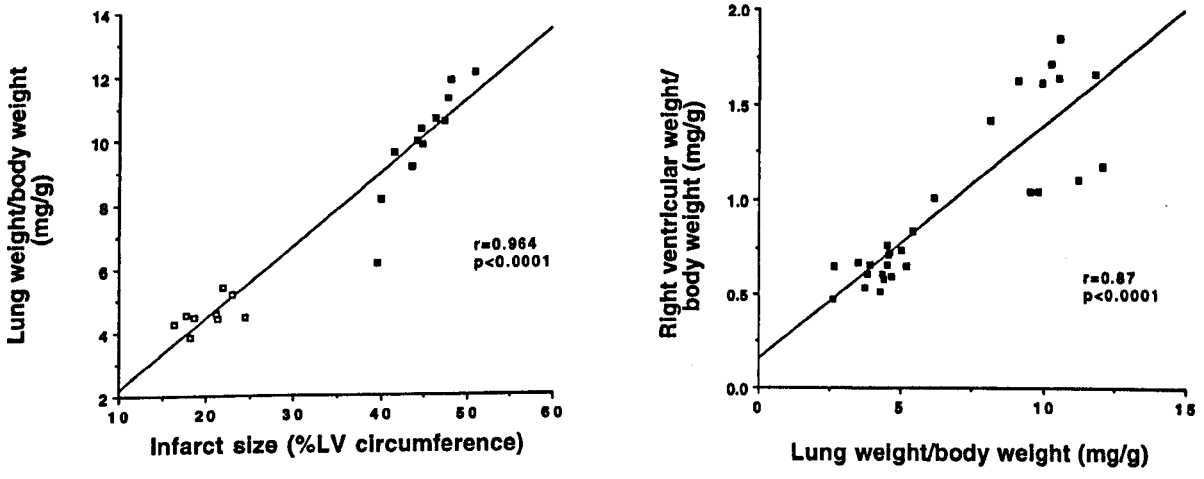

Figure 5.1. The relationship of lung wet weight/body weight ratio to infarct size expressed in % of left ventricular (LV) circumference which is infarcted in animals with congestive heart failure (black squares) and animals with left ventricular dysfunction (white squares) (left panel), and to right ventricular weight/body weight in infarcted and sham-operated rats (right panel). There are significant linear correlations.

and with the RV weight ($r=0.87$, $p<0.001$; Figure 5.1 right panel). As shown in Figure 5.1 (left panel) there was a clear separation between the groups with exact agreement in the allocation into congested with large infarcts and non-congested with small infarcts. The body weight was not significantly lower in the MI-HF animals (Table 5.1).

TABLE 5.1. Body Weight (BW), Right Ventricular Wet Weight/Body Weight (RVW/BW), Lung Weight/Body Weight (LW/BW), Infarct Size (I Size), Calf Weight/Body Weight (CW/BW) in Trained Rats with Myocardial Infarct and Left Ventricular Dysfunction (group 1), Trained Congestive Heart Failure (group 2), Non-Trained Left Ventricular Dysfunction (group 3), Non-Trained Congestive Heart Failure (group 4), Sham-operated (group 5).

Group	1	2	3	4	5
n	5	6	5	6	10
BW (g)	236±16	238±18	243±21	218±16	227±15
RVW/BW (mg/g)	0.66±0.13	1.35±0.29***	0.68±0.08	1.46±0.35***	0.62±0.08
Lung W/BW (mg/g)	4.85±0.53	10.53±1.50***	4.40±0.32	9.37±1.65***	3.83±0.88
I Size (%)	24.8±11.6	45.5±4.1***	20.7±2.5	44.3±2.6***	-
Calf W/BW (mg/g)	16.5±2.48	16.2±1.9	15.7±1.6	16.7±3.5	18.8±1.6*

Results are expressed as mean±standard deviation.
*p<0.05 cf. all the other groups, ***p<0.001 cf. appropriate training status left ventricular dysfunction group.

TABLE 5.2. Phosphorus-31 Magnetic Resonance Spectroscopy Measurements in Trained Rats with Myocardial Infarct and Left Ventricular Dysfunction (group 1), Trained Congestive Heart Failure (group 2), Non-trained Left Ventricular Dysfunction (group 3), Non-trained Congestive Heart Failure (group 4), Sham-operated (group 5).

Group	1	2	3	4	5
n	4	6	5	6	8
Rest					
PCr/(PCr+Pi)	0.90±0.02	0.90±0.02	0.91±0.03	0.89±0.04	0.91±0.03
pH	7.03±0.03	7.03±0.02	7.06±0.07	7.01±0.08	7.03±0.07
Tension developed during maximal stimulation					
Max T (g)	362±109	321±57	346±40	271±43	338±69
End T (g)	265±70	263±59	295±55	208±74	
End exercise					
PCr/(PCr+Pi)	0.54±0.13	0.53±0.09	0.52±0.08	0.40±0.14	0.54±0.10
pH	6.96±0.04	6.96±0.018	6.96±0.03	6.86±0.10*	6.96±0.04
200 g tension					
PCr/(PCr+Pi)	0.71±0.04	0.67±0.09	0.72±0.10	0.50±0.16**	0.71±0.11
pH	7.00±0.02	7.01±0.035	6.97±0.04	6.93±0.071*	6.99±0.03
Recovery					
PCr (T1/2) (min)	0.36±0.10	0.31±0.06	0.33±0.08	0.59±0.17**	0.29±0.05
ADP (T1/2) (min)	0.21±0.08	0.16±0.05	0.21±0.04	0.30±0.10*	0.20±0.07

Max T = Maximum twitch tension; End T = End stimulation twitch tension; 200 g = Normalised twitch tension at a force of 200 g.
* p<0.05, **p<0.01, cf. all other infarcted groups and sham-operated group where appropriate.

ii. Allocation of the Animals to the Different Groups

Among 100 operated rats, all the sham-operated survived (n=10), 53 of 90 infarcted rats died in the first 48 hours after the coronary ligation (59%) and 7 died in the first 6 weeks before randomisation (8%).

The remaining 30 animals were randomised 6 weeks after the procedure in two groups: the first group (16 animals) was trained and the second (14 animals) was not trained. The training period lasted over 6 weeks. Five animals died in the trained group and 3 in the non-trained group. These death rates between the different groups of infarcted rats were not significantly different.

The sciatic nerve was damaged in 2 sham operated animals and in 1 trained rat. Thus, the population of the nuclear magnetic resonance study comprised 10 trained rats with myocardial infarct, 11 non-trained rats with myocardial infarct and 8 sham-operated animals. The population of the enzyme assays comprised 11 trained, 11 non-trained and 10 sham animals. At the end of the experiments the animals were assigned to the different groups according to the lung wet weight/body weight ratio. Infarct sizes are shown in Table 5.1.

iii. Calf Muscle Mass and Maximum Attained Twitch Tension

The muscle mass expressed either as calf weight or as calf/body weight ratio was significantly higher in the sham animals than in the infarcted non-trained or trained

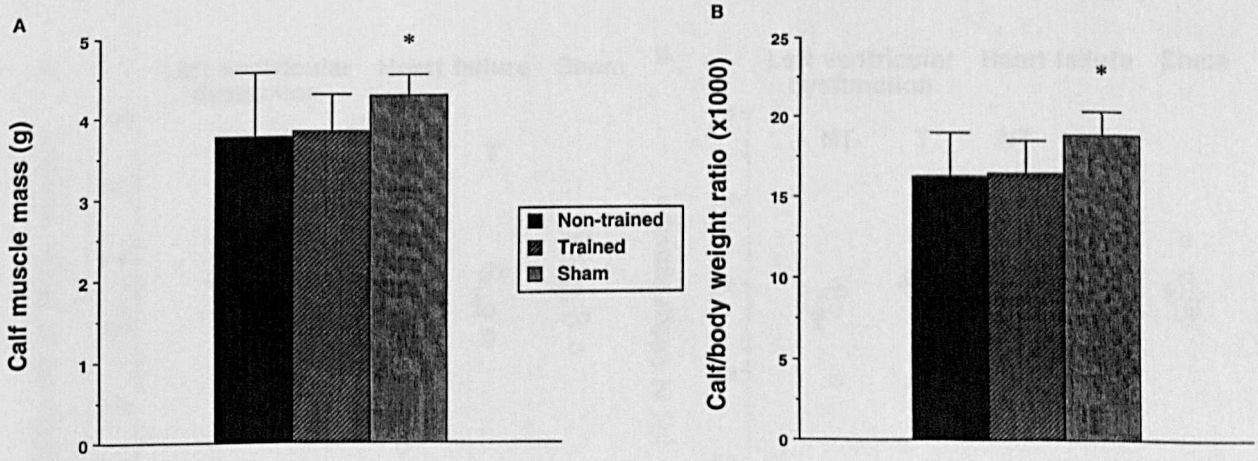

Figure 5.2. Comparisons of calf muscle mass in grams (A) and calf/body weight ratio (x1000) (B) between the three groups of animals: non-trained, trained and sham-operated; * $p<0.05$.

animals (Table 5.1 and Figures 5.2A and 5.2B). There was no difference in muscle mass or in maximal tension relative to calf mass between any of the infarcted group of animals and thus metabolic changes during exercise are examined between the infarcted groups only (Table 5.2).

iv. Magnetic Resonance Spectroscopy Results

1. *Rest.* There was no difference at rest between the five groups either for PCr/(PCr+Pi) ratio or for pH (Table 5.2).

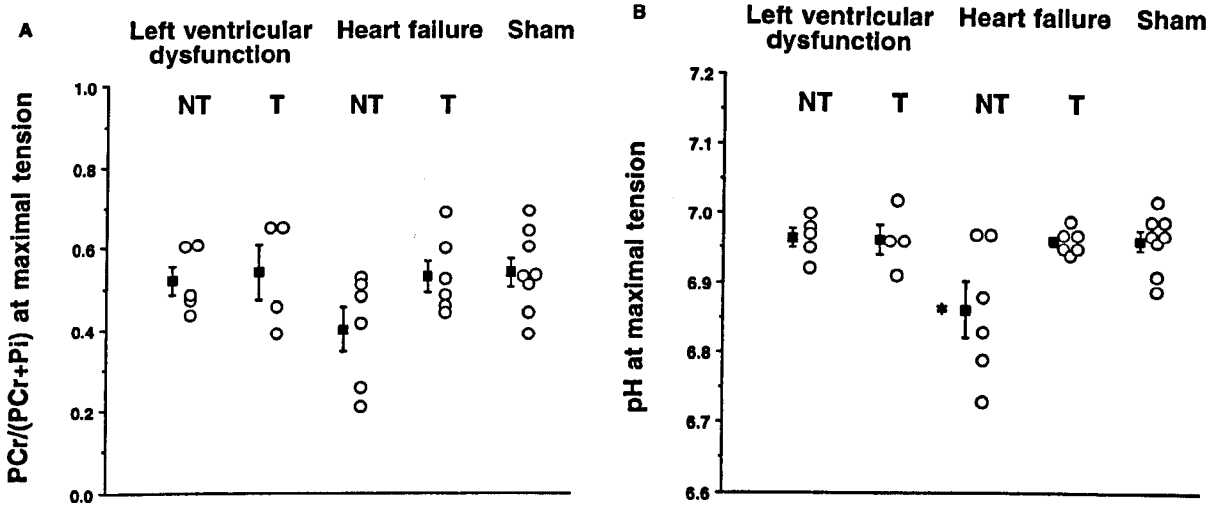

Figure 5.3. Minimum PCr/(PCr+Pi) ratio (A) and minimum pH (B) measured during the 640 secs of isometric contraction at the maximum attainable twitch tension. T = trained animals, NT = non-trained animals; * $p<0.05$.

2. *Sciatic nerve stimulation.* The only group of animals which behaved differently was the non-trained group with MI-HF, even though these animals had a lower maximal twitch

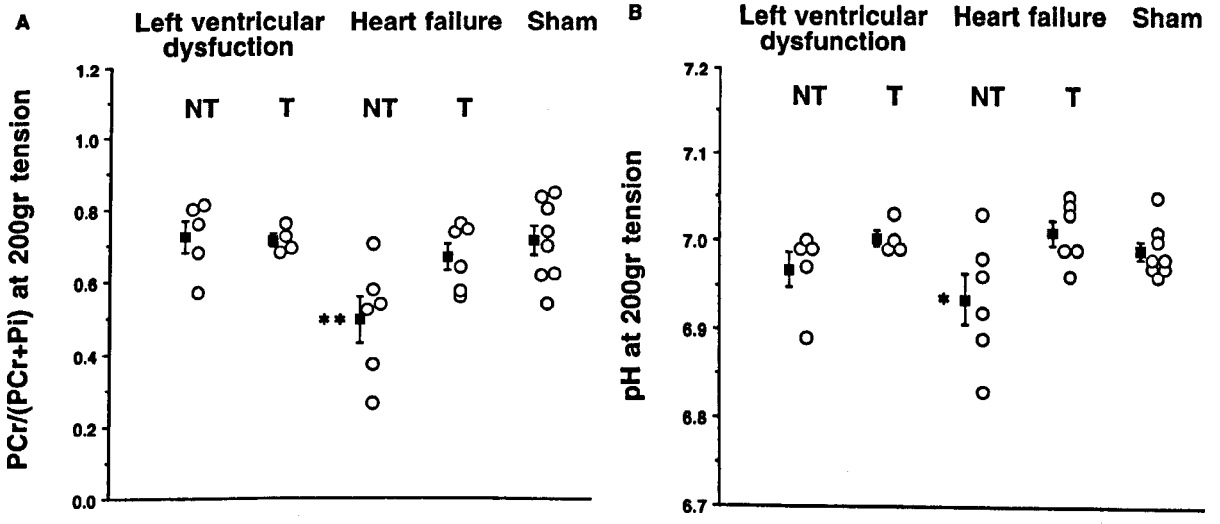

Figure 5.4. Minimum PCr/(PCr+Pi) ratio (A) and minimum pH (B) measured during the 320 sec of isometric contraction at 200 g twitch tension. T = trained animals, NT = non-trained animals; ** $p<0.01$, * $p<0.05$.

tension. The four other groups did not show any significant inter-group differences. At maximal twich stimulation a non-significant trend towards lower PCr/(PCr+Pi) was observed in non-trained MI-HF animals compared with all the other groups (Figure 5.3A). The end

exercise pH following maximal twich stimulation was significantly lower in the non-trained MI-HF group than in all the other groups, including the trained MI-HF group ($p<0.05$, Figure 5.3B).

After stimulating the leg with a constant twitch tension of 200 g for 320 sec (submaximal twitch tension), PCr/(PCr+Pi) was found to be lower in the non-trained MI-HF group compared with the other groups ($p<0.01$, Figure 5.4A) indicating significantly higher PCr utilisation in this group. Non-trained MI-HF animals also showed a significantly higher degree of acidification (lower pH) at this submaximal twich tension compared with all the other groups ($p<0.05$, Figure 5.4B).

3. *Recovery.* Recovery half-times for both PCr (Figure 5.5A) and ADP (Figure 5.5B) were significantly longer (ANOVA, $p<0.01$ and $p<0.05$ respectively) in the non-trained MI-HF

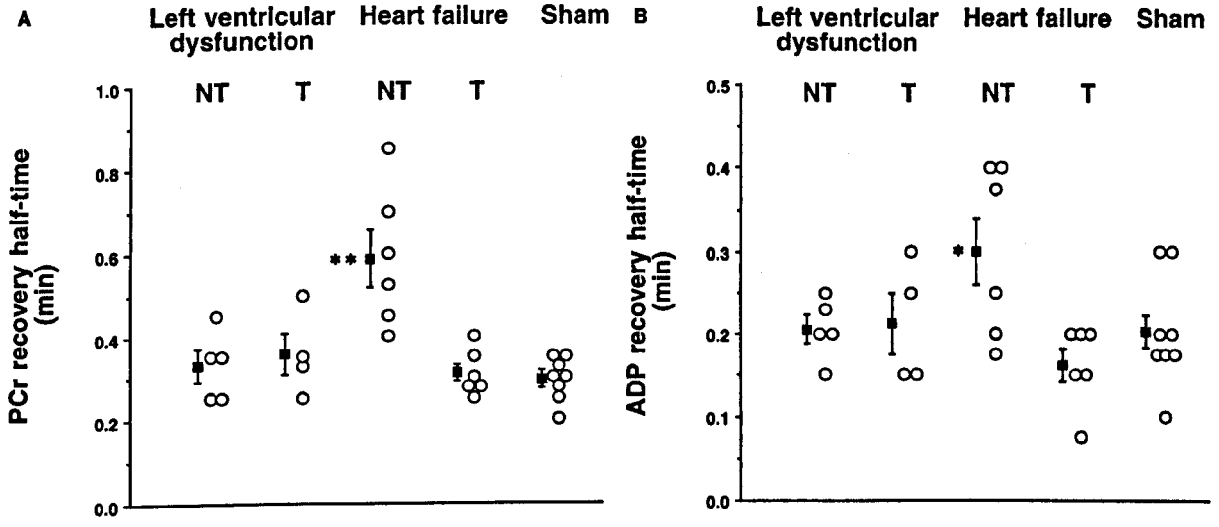

Figure 5.5. Phosphocreatine (PCr) (A) and adenosine diphosphate (ADP) (B) recovery half-time. Note the significantly longer recovery half-time for PCr and ADP in the non-trained congestive heart failure group; ** $p<0.01$, * $p<0.05$.

group compared with all the other groups. The trained MI-HF group showed recovery half-times similar to those observed in the sham-operated control group, indicating that exercise training reverses to normal the delayed PCr resynthesis and delayed restoration of ADP, seen in the non-trained MI-HF group.

v. Calf Muscle Histology

The proportion of fibre types in each group was similar. The fibre composition of rat hind limb muscle has been well described (Armstrong and Phelps, 1984) and it is known that fibre type prevalence varies in biopsies from different animals due to sampling errors. It was, thus, decided to compare fibre diameter for each fibre type as shown in Table 5.3. There was no significant difference in this sample between the individual fibre diameters of calf muscle from the trained, non-trained and sham operated animals or between the MI-HF and MI-LVD groups.

TABLE 5.3. Effects of Exercise Training on Fibre Size for Each Fibre Type Group

	Fibre size (μm)		
Fibre type	**Non-trained**	**Trained**	**Sham**
I	42.8±3.9	40.1±5.9	48.1±0.6
IIa	39.9±2.3	42.6±1.1	49.7±4.6
IIb	45.5±4.5	48.3±3.6	41.6±1.3
All type II	43.9±4.0	45.7±2.2	45.6±2.6

vi. Enzymatic Assays

The non-trained MI-HF animals exhibited a reduced activity of the mitochondrial enzyme citrate synthase (Table 5.4). Citrate synthase activity in the trained animals with congestive heart failure was found at the same level as shams (Figure 5.6 left panel). ß-Hydroxyacyl CoA dehydrogenase activity was also modified in the same way but not significantly. The glycogenolytic enzyme phosphorylase was not significantly modified in any group. When compared with the other groups glutamate pyruvate transferase activity demonstrated an increased activity in the trained MI-HF group which did not reach significance. The activity of this enzyme was increased by training alone (significant training effect, $p<0.05$ by ANOVA, but there was no significant effect of infarct size; Figure 5.6 right panel).

TABLE 5.4. Enzyme Assays in Trained Rats with Myocardial Infarct and Left Ventricular Dysfunction (group 1), Trained Congestive Heart Failure (group 2), Non-trained Left Ventricular Dysfunction (group 3), Non-trained Congestive Heart Failure (group 4), Sham-operated (group 5).

Group	1	2	3	4	5
n	5	6	5	6	10
Citrate synthase	23.5±4.0	24.9±1.8	21.0±2.5	18.3±2.0**	25.9±3.8
BOAC	2.2±0.5	2.7±0.3	1.9±0.9	2.0±0.5	2.9±1.4
Phosphorylase	86±13	87±31	87±15	84±10	85±12
GPT	7.37±1.5	8.38±0.7	6.69±1.0	6.41±1.5	7.13±1.1

BOAC = ß-Hydroxyacyl CoA dehydrogenase; GPT = Glutamate pyruvate transferase.
Results are expressed in $\mu mol.min^{-1}$ / g wet weight at 30° C as mean±standard deviation. ** $p<0.01$ cf. groups 1,2 and 5.

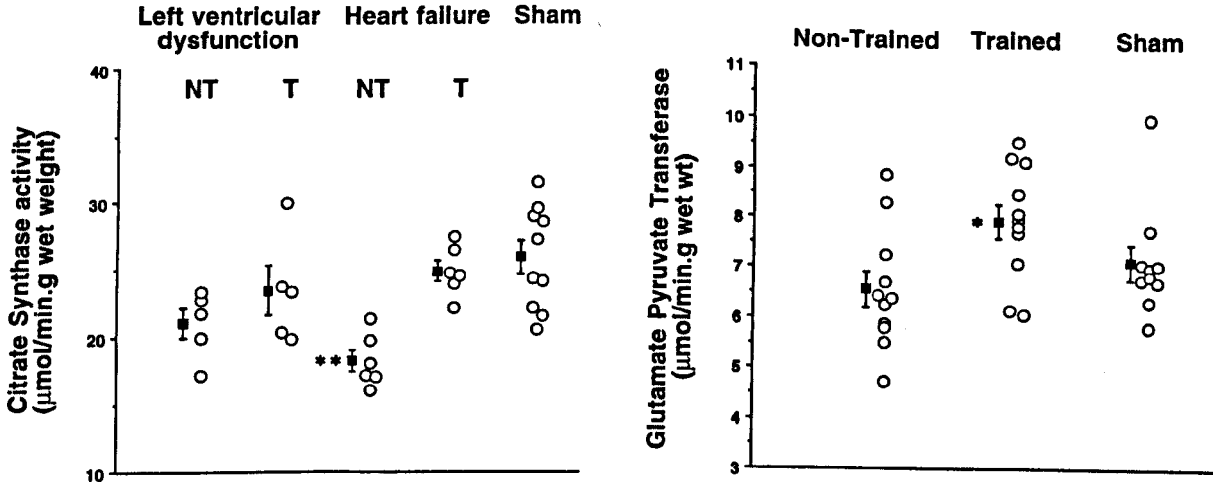

Figure 5.6. Muscular citrate synthase activity (expressed in µmol/min.g wet weight) in the five groups of animals (left panel) and muscular glutamate pyruvate transferase activity (expressed in µmol/min.g wet weight) in the three groups of animals (right panel). T = trained animals, NT = non-trained animals; * $p<0.05$, ** $p<0.01$

vii. Correlations

There was a significant inverse correlation between citrate synthase activity and PCr recovery half-time ($r=-0.58$, $p<0.001$; Figure 5.7) suggesting that reduced mitochondrial

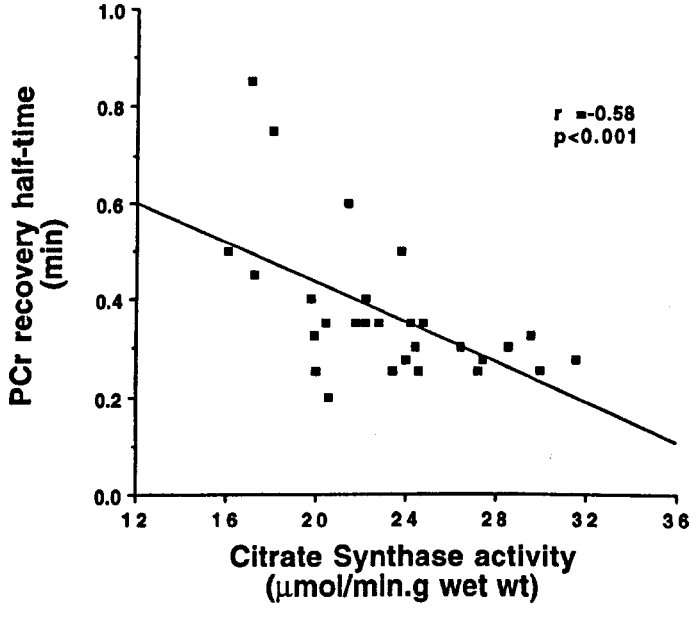

Figure 5.7. The relationship of the half-time of phosphocreatine (PCr) recovery and the citrate synthase activity of the hindlimb of both infarcted and sham-operated rats. There is a significant linear (inverse) correlation.

oxidative capacity accounts significantly for the MRS changes in the calf muscle. A good correlation was also found between infarct size and PCr recovery half-time in the non-trained group ($r=0.68$, $p<0.03$).

E. Discussion

This study demonstrates that the rat with congestive heart failure develops similar skeletal muscle metabolic changes in the handling of high energy phosphates during exercise to those described in heart failure in humans (Massie *et al.*, 1987b ; Arnolda *et al.*, 1990). The most important conclusion emerging from this study is that skeletal muscle metabolic abnormalities seen in congestive heart failure can be modified or corrected by physical training; physical training may even prevent these abnormalities. In infarcts of the size reported here, changes in skeletal muscle metabolism are present 4-6 weeks after infarction (Arnolda *et al.*, 1991; Thompson *et al.*, 1995) yet these changes are absent 12 weeks after infarction following 6 weeks of training. This study also provides evidence that alterations in oxidative capacity of skeletal muscle could provide the biochemical basis of these MRS abnormalities in both the trained and the untrained state and the metabolic benefit of training occurs despite the persistence of muscle atrophy.

i. Effects of Heart Failure on Skeletal Muscle

Our experiments were conducted in an established model of heart failure, that produced by myocardial infarction in rats (Pfeffer *et al.*, 1979; Fletcher *et al.*, 1981; Hostetter *et al.*, 1983; Pfeffer *et al.*, 1985; Drexler *et al.*, 1987). We used the criterion of lung weight/body weight ratio (Fletcher *et al.*, 1981; Hostetter *et al.*, 1983; Pfeffer *et al.*, 1985) to grade rats with infarcts according to the severity of their left ventricular dysfunction. Thus, we separated rats with infarcts, regardless of their training status, into congestive heart failure (MI-HF) and left ventricular dysfunction (MI-LVD) groups. Infarct sizes in the MI-HF group ranged from 40-52% (mean 45%) and in MI-LVD group from 16-26% (mean 21%). It has been repeatedly demonstrated that infarcts of the size observed in MI-HF group (≥40%) are associated with markedly elevated left ventricular end-diastolic pressure, elevated right sided filling pressures and reduced cardiac output at rest and with grossly impaired cardiac responses to increased preload and afterload (Pfeffer *et al.*, 1979; Fletcher *et al.*, 1981; Hostetter *et al.*, 1983; Pfeffer *et al.*, 1985). The highly increased RV weight/body weight ratio in this group, which reflects elevated left ventricular end-diastolic pressure in the MI-HF group, and the high correlation between infarct size and lung weight/body weight or RV weight/body weight ratio in our study provides further confirmation of the severity of heart failure in the MI-HF group. We might reasonably expect that in infarcts of the sizes reported here the congestive changes of heart failure were present from at least 3-4 weeks post infarct until the rats were killed (Pfeffer et al., 1979; Arnolda *et al.*, 1991; Thompson *et al.*, 1995). Since significant exercise and recovery abnormalities were observed in the untrained MI-HF group and not the untrained MI-LVD group, this suggests that the severity of heart failure has some importance in defining the metabolic defects in skeletal muscle.

In the present study, we added an additional stimulation protocol in our animals in which we adjusted the stimulation parameters to produce a tension of 200 g and then followed changes in metabolites during sciatic nerve stimulation. This was done to avoid the

variation in tension produced in the maximal stimulation protocol due to changes in local collection of fluid around the nerve and electrodes. The 200 g protocol indeed produced much clearer differences between the groups as predicted. In addition, it is possible that the first maximal test had made the muscle more sensitive to the second.

It has been suggested that the changes in skeletal muscle metabolism in heart failure could be secondary to atrophy (Buller *et al.*, 1991) or to alteration in the proportion of glycolytic to aerobic fibres within the muscle. We have been able to exclude a major contribution from either of these. Muscle atrophy is a frequent finding in heart failure (Mancini *et al.*, 1992b) and it has been shown in human studies that atrophy makes a contribution to the exercise intolerance of the patient with heart failure. We found a reduction in calf muscle weight in infarcted rats but it was no different in MI-HF and MI-LVD groups and for the same tension (200 g), PCr and pH changes during exercise were greater in the MI-HF group. Also the maximal tension expressed relative to the calf mass was not different in any group, suggesting that the work performed was similar at maximal tension. This suggests that muscle atrophy cannot be responsible for all the metabolic changes seen in heart failure.

Inorganic phosphate, PCr concentration and pH are normal in resting muscle in these rats with heart failure. If there has been a major change in the proportion of fibre types in the muscle, the resting muscle spectra would have appeared abnormal (Meyer *et al.*, 1985; Kushmerick *et al.*, 1992). Histological examination also failed to reveal any training-induced alteration in the proportion of fibre types of the infarcted rats. A large change in the proportion of fibre types in these muscles is unlikely.

The results in the non-trained rats confirm the findings reported previously from our group that though PCr/(PCr+Pi) and pH are normal in resting muscle in rats with heart failure compared with control animals (Arnolda *et al.*, 1991), during exercise there is a greater utilisation of PCr and a greater fall in pH in the infarcted animals. These changes were paralleled by reduction in citrate synthase in both studies. In addition, the severity of changes were greater in rats with the bigger infarcts in both studies. In the present study, the changes in the animals with small infarcts (left ventricular dysfunction), showed changes in the same direction as those with larger infarcts, but the differences did not reach statistical significance with the number of animals studied. The MRS results were not significant for PCr/(PCr+Pi) ratio, possibly because the maximal work was less for the non-trained congestive animals. Clearer differences were seen for a matched (200 g) workload.

A better insight in the biochemical substrate of the training-induced ^{31}P MRS changes in infarcted rats is provided by the findings in enzymatic activity in our experimental model. Citrate synthase, a mitochondrial oxidative enzyme, was markedly reduced in skeletal muscle in non-trained rats with large myocardial infarcts. The muscle wet/dry weight ratio does not alter after myocardial infarction (49% of left ventricular circumference) (Thompson *et al.*, 1995), so this is probably a true reduction in citrate synthase activity. Decrements, also, in mitochondrial emzyme activities (citrate synthase and phosphofructokinase) independent of muscle fibre composition have been recently observed (Delp *et al.*, 1997) in

rats with severe left ventricular dysfunction after myocardial infarction. Skeletal muscle glycogenolysis is increased in rats following myocardial infarction (Musch *et al.*, 1990) and together with reduction of the activity of the enzymes involved in oxidative metabolism, there could be greater lactic acid production contributing to the increased acidification of the skeletal muscle during the sciatic nerve stimulation. The rate of PCr resynthesis during recovery has been established as an index of oxidative metabolism independent of muscle mass or workload (Mancini *et al.*, 1992b). The significant correlation found between citrate synthase levels and PCr recovery half-time further supports the hypothesis that the reduced mitochondrial oxidative capacity and the increased anaerobic ATP synthesis could be responsible for the changes detected by ^{31}P MRS (increased PCr utilisation and acidification during stimulation and the impaired PCr resynthesis during the recovery) in the infarcted non-trained rats. Recent investigations provide new insight into the contribution of mitochondria to intrinsic skeletal muscle alterations in CHF; reduction in mitochondrial creatine kinase (a key enzyme for rapid energy transfer from mitochondria to cytosol) is associated with increased expression of inducible NO synthase (iNOS) in the skeletal muscle, suggesting that NO produced by iNOS attenuates mitochondrial energy transfer and thus, possibly, explaining the delayed resynthesis of PCr and early muscular fatigue during exercise observed in CHF (Gross *et al.*, 1996; Hambrecht *et al.*, 1999).

ii. The Effect of Training on Skeletal Muscle Metabolism in Heart Failure

In the MI-HF animals, the training protocol used in this study normalised citrate synthase activity as well as the exercise and recovery metabolic abnormalities. This suggests that training increased oxidative metabolism in these muscles. The training of the animals could not have been intensive otherwise a marked incerase in the activities of citrate synthase, ß-hydroxyacyl CoA dehydrogenase and glutamate pyruvate transferase would have been observed in the trained group of animals with a small infarct (Mole *et al.*, 1973; Holloszy, 1976).

In the present study, training could not reverse the skeletal muscle wasting (Table 1), again suggesting that muscle atrophy was not an important factor in these metabolic changes. This finding is in agreement with a study showing improvement in skeletal muscle energetics in the forearm after training without alterations in muscle mass or limb blood flow (Minotti *et al.*, 1990b). Muscle atrophy may, however, have a greater impact on exercise intolerance, fatigue and maximal muscle blood flow (Ludman *et al.*, 1993; Volterrani *et al.*, 1994). Thus, the normalisation of muscle metabolism achieved by training in rats without affecting the skeletal muscle wasting may explain our findings in a human study that physical training can reverse the MRS-described muscle metabolic abnormalities but can only very partially correct the limiting fatigue and exercise intolerance in heart failure patients (Adamopoulos *et al.*, 1993).

The only group which has abnormal muscle metabolism is the group of MI-HF rats who have not been trained. This finding suggests that the observed abnormalities are not entirely the result of the heart failure itself [and potential neuroendocrine factors, blood flow etc.

(Drexler *et al.*, 1987)] but also of muscular inactivity. During rat hindlimb suspension, changes in fibre composition of the soleus occur with transformation of type I to type II (Templeton *et al.*, 1988). Physical inactivity results in decreased enzyme activity and training has been shown to increase citrate synthase and ß-hydroxyacyl CoA dehydrogenase activity (Holloszy, 1976). Our results could be explained by a training-induced increase in oxidative metabolism, which could reduce PCr depletion and lactate production and accumulation, and also speed PCr and ADP recovery after stimulation. This is supported by the normal activity found for citrate synthase after training in the animals with CHF. Rats with heart failure responded to a physical training programme by increasing (or normalising) not only levels of the mitochondrial oxidative enzymes citrate synthase and ß-hydroxyacyl CoA dehydrogenase but also levels of the mitochondrial-cytoplasmic enzyme glutamate pyruvate transferase. Training is known to induce an increase of the level of glutamate pyruvate transferase, which permits the generation of alanine and ketoglutarate from pyruvate and glutamate, thus reducing the formation of lactate during exercise and hence limiting the fall in the pH, providing at the same time more oxaloacetate to the first step of Krebs cycle (Mole *et al.*, 1973; Holloszy, 1976). The increased glutamate pyruvate transferase activity in the congestive trained group might suggest a different biochemical mechanism explaining the improvement in skeletal muscle abnormalities with physical training and could explain, at least partially, the increase in exercise capacity in our heart failure patients (Coats *et al.*, 1992a). These results are consistent with another study of heart failure showing that skeletal muscle succinate dehydrogenase activity is greater and that the blood lactic response to exercise is lower in trained rats compared with sedentary rats (Musch *et al.*, 1986). The normal phosphorylase concentration we have found in the five groups of animals studied seems to indicate that the glycogenolysis is normal in animals with heart failure.

The importance of altered blood flow to skeletal muscle in heart failure is not established. In heart failure in humans the abnormal MRS findings in muscle were not associated with abnormal blood flow during submaximal exercise (Massie *et al.*, 1987a and 1988). Moreover, chronic ischaemia and/or hypoxia induces an increase in oxidative enzyme activities both in rats and in humans (Holm *et al.*, 1972 a&b; Bylund *et al.*, 1976; Elander *et al.*, 1985) in contrast to the opposite metabolic changes observed in heart failure. Training alters the lactate production in exercise independently of the improvements in blood flow (Sullivan *et al.*, 1988a). Nevertheless, it is possible that the metabolic effects of training in these rats could be the result of an increase in substrate supply to the muscle owing to altered blood flow to the exercising leg. Any increased blood flow may also prevent lactate accumulation inside the cell by promoting efflux of lactate down an increased transmembrane concentration gradient. Other studies of this rat model of heart failure demonstrate changes in leg blood flow. The leg muscle blood flow during exercise was lower in an infarcted group (Musch and Terrell, 1992b) with blood flow selectively decreased to oxidative muscles (Drexler *et al.*, 1987). Blood flow to the rat hindlimb may be decreased

even further following training with a relative increase in flow to the oxidative muscles (Musch *et al.*, 1992a).

In conclusion, the increased metabolic tolerance to electrical stimulation in the trained animals with congestive heart failure does not seem to be related to an increase in the muscle mass or to a change in the relative proportion or hypertrophy of any type of muscle fibre. The metabolic changes may be an effect of deconditioning of the muscle and the trend towards more glycolytic activity in skeletal muscle in CHF (Musch *et al.*, 1990). The correction of these changes after training could be explained by the previously reported differences in blood flow distribution towards glycolytic muscles prior to training (Drexler *et al.*, 1987) and towards oxidative muscles following training (Musch *et al.*, 1992a).

Our study shows that physical training is able to prevent or even correct abnormalities of skeletal muscle metabolism after experimental infarction in the rat. The metabolic changes in rat in CHF seem to reflect an adjustment to a state of lower physical activity yet are separate to the muscle atrophy seen in this condition. These experimental data support the hypothesis that cardiac rehabilitation after myocardial infarction could be useful in improving muscle oxidative capacity in the case of severely altered cardiac function.

Chapter VI

Physical Training Improves Skeletal Muscle Metabolism in Patients with Chronic Heart Failure

A. Abstract

Objectives and Background. This study investigated the effects of physical training on skeletal muscle metabolism in patients with CHF. Skeletal muscle metabolic abnormalities in patients with CHF have been associated with exercise intolerance. Muscle deconditioning is a possible mechanism for the intrinsic skeletal muscle metabolic changes seen in CHF.

Methods. We used ^{31}phosphorus magnetic resonance spectroscopy (^{31}P MRS) to study muscle metabolism during exercise in 12 patients with stable ischaemic CHF undergoing 8 weeks of home based bicycle exercise training in a randomised crossover controlled trial. Changes in muscle pH and in the concentrations of phosphocreatine (PCr) and adenosine diphosphate (ADP) were measured in ^{31}P spectra of calf muscle obtained at rest, throughout incremental-workload plantarflexion until exhaustion and during recovery from exercise. Results were compared with those in 15 age-matched controls, who performed a single study only.

Results. Before training, PCr depletion, muscle acidification and rise in [ADP] during the first 4 min of plantarflexion were all increased ($p<0.04$) compared with the corresponding values in control subjects. Training produced an increase ($p<0.002$) in incremental plantarflexion exercise tolerance. After training, PCr depletion and the rise in [ADP] during exercise were reduced significantly ($p<0.003$) at all matched submaximal workloads and at peak exercise, although there was no significant change in the response of muscle pH to exercise. After training, changes in [ADP] were not significantly different from those in control subjects, although PCr depletion was still greater ($p<0.05$) in trained patients than in controls. The PCr recovery half-time was significantly shorter after training ($p<0.05$), although there was no significant change in the half-time of ADP recovery. In untrained subjects, the initial rate of PCr resynthesis after exercise [a measure of the rate of oxidative adenosine 5'-triphosphate (ATP) synthesis] and the inferred maximal rate of mitochondrial ATP synthesis were reduced compared with rates in control subjects ($p<0.003$) and both were significantly increased by training ($p<0.05$) so that they were not significantly different from values in control subjects.

Conclusions. The reduction in PCr depletion and in the rise of [ADP] during exercise, and the enhanced rate of PCr resynthesis in recovery (which is independent of muscle mass) indicate that a substantial correction of the impaired oxidative capacity of skeletal muscle in CHF can be achieved by exercise training.

B. Introduction

Exercise intolerance is a major limiting symptom in heart failure and has been attributed to skeletal muscle hypoperfusion during exercise as a result of both diminished cardiac output and impaired vasodilatory reserve (Zelis *et al.*, 1975; Wilson *et al.*, 1984b; Sullivan *et al.*, 1989b).

Interventions which improve haemodynamics and left ventricular function do not alter exercise capacity immediately. The failure of exercise capacity or skeletal muscle oxygen utilisation to rise acutely when peripheral blood flow is increased (Wilson *et al.*, 1983a and 1984a) suggests that intrinsic abnormalities of skeletal muscle also play a role in the pathophysiology of exertional fatigue in CHF. In contrast, chronic vasodilator therapy can produce significant increases in exercise performance (Franciosa *et al.*, 1980), suggesting that any such intrinsic muscle abnormality can eventually be reversed.

Studies using ^{31}P MRS have demonstrated early and excessive acidification and PCr depletion in skeletal muscle in patients with CHF (Massie *et al.*, 1987b and 1988). These MRS changes could not be explained by alterations in blood flow (Wiener *et al.*, 1986; Massie *et al.*, 1988), suggesting an intrinsic abnormality in skeletal muscle metabolism. Other investigators (Mancini *et al.*, 1989; Sullivan *et al.*, 1990) have shown major alterations in skeletal muscle histology and biochemistry in patients with long-term CHF, including fibre atrophy, transformation of type I to type II fibres and a decrease in oxidative enzyme capacity.

There are many similarities between the abnormalities associated with CHF and those seen in physical deconditioning (Adamopoulos *et al.*, 1992). In both conditions there is exercise intolerance, sympathetic hyperactivity, wasted skeletal muscles, decreased fibre size and depleted skeletal muscle oxidative enzymes (Green *et al.*, 1980; Coyle *et al.*, 1984). Physical training programmes improve exercise performance, ventilation, autonomic function and symptomatic status in CHF (Sullivan *et al.*, 1988a; Coats *et al.*, 1990 and 1992a). We have previously reported on the beneficial effects of physical training in reversing the skeletal muscle metabolic changes in experimental heart failure in rats (Brunotte *et al.*, 1995) and two relatively recent studies reported beneficial effects from single arm training and from leg muscle training on a bicycle ergometer (Minotti *et al.*, 1990b; Hambrecht *et al.*, 1995). There has been no controlled trial, however, on the effect of endurance training on leg muscle metabolism in patients with CHF by using non-invasive techniques.

In this study we used ^{31}P MRS to investigate the effects of physical training on skeletal muscle metabolic abnormalities in patients with CHF in a randomised controlled crossover trial, comparing home-based exercise training with activity restriction. We also investigated whether the skeletal muscle metabolism of patients with CHF after training is similar to that in age-matched normal control subjects.

C. Methods

i. Study Population

Twelve subjects gave informed consent for this trial which was approved by the local ethics committee. We studied only patients with stable CHF secondary to ischaemic heart disease and without angina or significant ventricular arrhythmias.

Patients were aged 62.4±2.6 (range 43-75 years). Seven were NYHA functional class II and 5 were in NYHA class III. Radionuclide left ventricular ejection fraction was 24±3.4% and peak oxygen consumption 12.1±1.2 ml/kg/min (mean±SEM). Four had undergone coronary artery by-pass grafting. All subjects were taking diuretics (median frusemide dose 80 mg); ten out of 12 were taking angiotensin-converting enzyme inhibitors. Pharmacological treatment was stable for 3 months prior to study and for the duration of the study in all subjects. Upon entry to the trial, patients underwent a 2- to 4-week familiarisation and baseline evaluation phase, during which reproducible exercise tests were obtained. Subsequently, all patients were randomised to 8 weeks bicycle exercise or avoidance of exercise in a crossover design, that has been previously described by us (Coats *et al.*, 1990). Our control group consisted of 15 age-matched healthy males aged 55.2±2.8 years (range 33-68).

ii. Exercise testing

Exercise tests were performed on a Tunturi Professional Ergometer (Tunturi, Finland). The upright bicycle tests were performed in 5-min stages, with 25-W increments to the limit of tolerance. All tests were performed before daily medication had been taken and were conducted by a neutral observer who had no knowledge of patient data. Oxygen consumption and CO_2 production were measured during the test (Coats *et al.*, 1990). On the same day, after 1 hour of rest, a second exercise test was performed in the supine position, during which pulsed wave Doppler ultrasonic measurements of ascending aortic blood velocity from the suprasternal approach were made at rest and at the end of each 5-min 25-W incremental stage of supine bicycle exercise test. Using a Pedof Doppler ultrasound generator (Vingmed, Norway) and our own laboratory made computer-based Fast Fourier Transform spectral analyser (Murphy *et al.*, 1988) stroke distance (the integral of velocity and time for the ejection period) was calculated; using standard formulae and an echocardiographic measurement of aortic cross-sectional area (leading edge to leading edge, immediately distal to the sinus of Valsalva), stroke volume was then calculated.

iii. Phosphorus-31 Magnetic Resonance Spectroscopy

Exercise was performed at least 4 hours after eating. The details of the ^{31}P spectroscopy protocol are as we have previously described (Arnolda *et al.*, 1990). Briefly, the patient lay supine with his right calf muscle placed over a 6 cm diameter surface coil in a 1.9 T 60 cm bore superconducting magnet (Oxford Magnet Technology, Oxford, England) interfaced to a

Bruker Biospec spectrometer (Coventry, UK). Phosphorus-31 spectra were acquired at 32.7 MHz using an interpulse delay of 2 sec. Pulse length was 60° (a 90° pulse at coil centre was 80 μsec).

Both normal control subjects and patients with heart failure patients were studied at rest, during 0.7 Hz (40/min) plantarflexion until exhaustion and during recovery from exercise. A resting spectrum was collected as the sum of 128 scans. Thirty-two scans were accumulated for each exercise spectrum. Exercise was performed using a pedal connected via pulleys to an adjustable weight. The initial workload was set at 1.5-W and 4 exercise spectra (64 sec each) were collected at this workload. Thereafter, the workload was increased by 0.5-W after each spectrum had been collected. Recovery was followed for approximately 10 min in which time 4 spectra each of 16 scans (32 sec per spectrum) were collected at first and thereafter 8 spectra of 32 scans were summed for each of 8 spectra (64 sec per spectrum). The time-averaged free induction decays were apodised, subjected to exponential multiplication to yield a line broadening of 6 Hz and Fourier transformed. The relative concentrations of inorganic phosphate (Pi), PCr and the ß-phosphate of ATP were determined by triangulation and corrected for differential saturation. Changes in PCr concentration were expressed by the PCr/(PCr+Pi) ratio; pH was calculated by the chemical shift difference between Pi and PCr (Taylor *et al.*, 1983). The cytosolic free concentration of ADP was calculated from the phosphocreatine/ATP ratio and pH, using the equilibrium constant for creatine kinase reaction (Taylor *et al.*, 1983). Recovery half-times for PCr and ADP were calculated by graphic interpolation. The initial rate of PCr resynthesis after exercise was calculated from the PCr concentration during the last exercise (t = 0) and first recovery (midpoint = 0.27 min) spectra. This is a direct estimate of the rate of mitochondrial ATP synthesis and normally has a hyperbolic relation to cytosolic ADP (Kemp *et al.*, 1993a). We, therefore, used the initial rate of PCr resynthesis and the [ADP] at the end of exercise to calculate an apparent maximal rate of mitochondrial ATP synthesis (that is the rate when ADP concentration is very high), making the assumption that the mitochondrial Km for ADP (that is the ADP concentration at which the oxidation rate is half-maximum) is not altered.

iv. Statistical Analysis

Statistical analysis was carried out according to the recommendations of Hills and Armitage for crossover trials (Hills and Armitage, 1979). Analysis of variance (ANOVA) for repeated measures was used to detect the differences in terms of PCr utilisation, acidification (pH) and [ADP] during exercise before and after physical training. Repeated measures ANOVA was also used to analyse sequential changes at rest and during the part of the exercise completed by both control subjects and patients after physical training. The differences in recovery variables before and after training were assessed by the Wilcoxon signed-rank test and differences from control subjects by the Mann-Whitney U test. All values were expressed as mean±SEM.

D. Results

An example of the training-induced changes in PCr utilisation at the same submaximal workload (2-W) for one of our subjects is depicted in Figure 6.1. There were no differences in muscle at rest with respect to muscle pH and phosphorus metabolite ratios (for example, PCr/ATP) in trained, detrained and control groups.

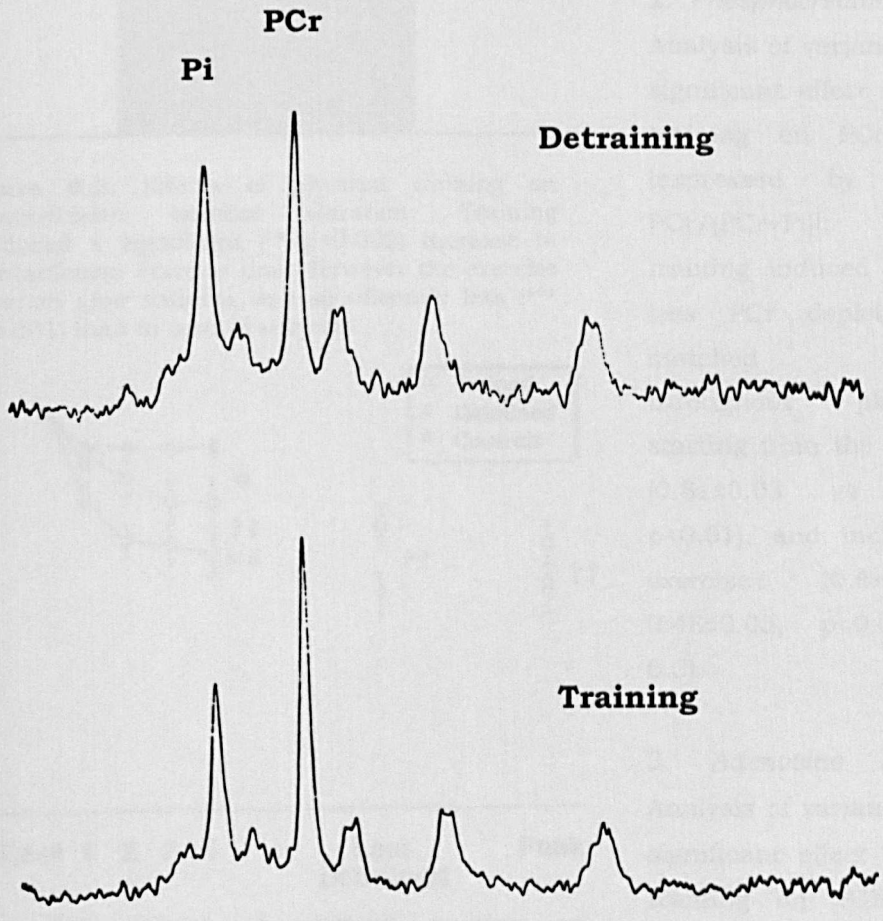

Figure 6.1. Phosphorus-31 nuclear magnetic resonance spectra of calf muscle during exercise at 2-W workload from a patient with chronic heart failure. From left to right, peaks are inorganic phosphate (Pi), phosphocreatine (PCr) and the γ–, α– and β–phosphates of ATP. Intracellular pH was calculated from the chemical shift difference between the Pi and PCr peak. Upper panel shows spectrum obtained after detraining and lower panel spectrum obtained after training during plantarflexion exercise at 2-W. Phosphocreatine is higher and Pi lower after physical training at the same workload

i. Effects of Physical Training During Plantarflexion

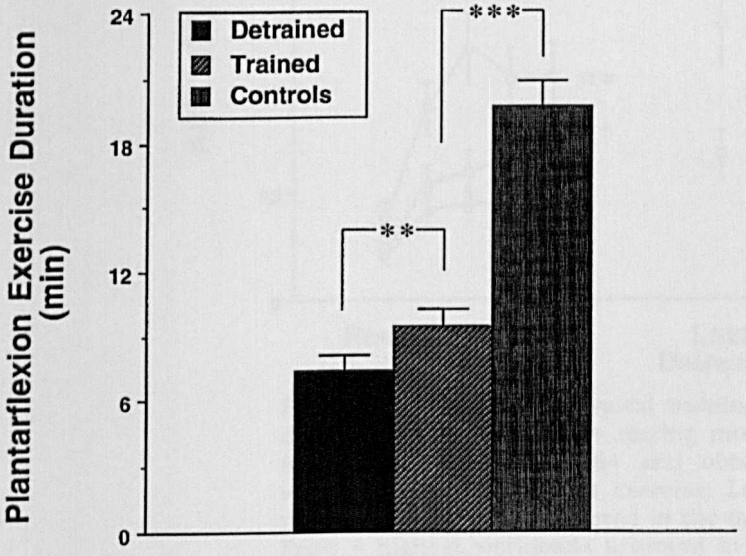

Figure 6.2. Effects of physical training on plantarflexion exercise duration. Training produced a significant (** p<0.002) increase in plantarflexion exercise time. However the exercise duration after training was significantly less (*** p<0.001) than in control subjects.

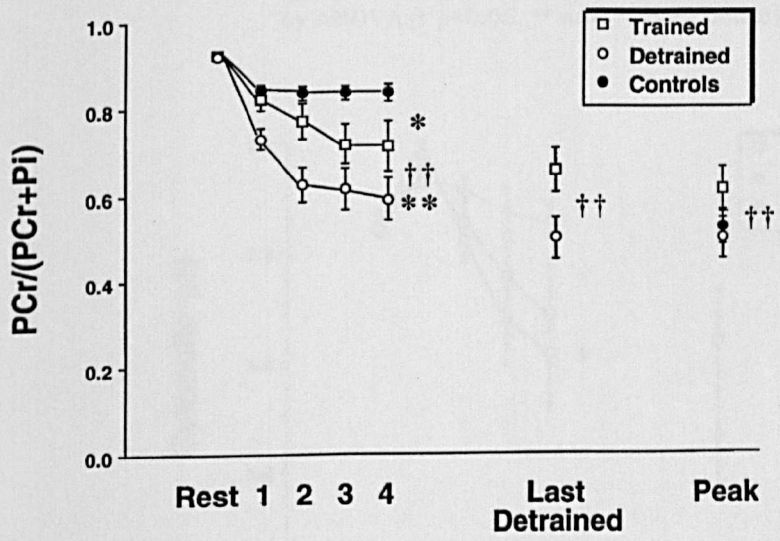

Figure 6.3. Effects of physical training on phosphocreatine (PCr) utilisation. Rest = resting muscle; 1-4 = the first four spectra (each 64 sec) obtained at 1.5-W workload of plantarflexion exercise; Last detrained = the highest workload achieved in the detraining study; Peak = highest workloads achieved in detraining and training studies; open squares = trained patients; open circles = detrained patients; closed circles = control subjects. Phosphocreatine depletion during exercise is significantly improved by training at all matched submaximal workloads and even at peak exercise. * and ** = comparison by ANOVA of detraining and training (respectively) studies with control subjects; †† = comparison of training with detraining studies by ANOVA (* p<0.05, ** and †† both p<0.01).

1. *Exercise tolerance.* There was a small but significant improvement in plantarflexion exercise tolerance. Plantarflexion exercise time was 7.4±0.7 min before training, increasing to 9.4±0.8 min after training (p<0.002) (Figure 6.2).

2. *Phosphocreatine utilisation.* Analysis of variance showed a significant effect (p<0.002) of training on PCr utilisation [expressed by the ratio PCr/(PCr+Pi)]; physical training induced significantly less PCr depletion at all matched workloads throughout plantarflexion, starting from the first minute (0.81±0.03 vs 0.72±0.02, p<0.01), and including peak exercise (0.6±0.05 vs 0.48±0.05, p<0.01) (Figure 6.3).

3. *Adenosine diphosphate.* Analysis of variance showed a significant effect (p<0.003) of training on [ADP]; physical training produced significantly smaller rise in [ADP] at all matched workloads throughout plantarflexion, starting from the first minute (19.6±4.5 vs 34.9±5.1 μM, p<0.01), and including peak exercise (30.8±4.3 vs 51.1±7.8 μM, p<0.05) (Figure 6.4).

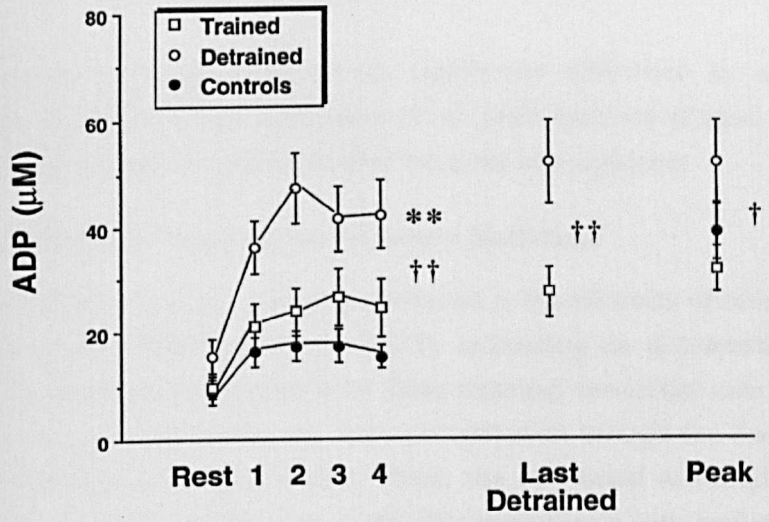

Figure 6.4. Effects of physical training on adenosine diphosphate (ADP). Rest = resting muscle; 1-4 = the first four spectra (each 64 sec) obtained at 1.5-W workload of plantarflexion exercise; Last detrained = the highest workload achieved in the detraining study; Peak = highest workloads achieved in detraining and training studies; open squares = trained patients; open circles = detrained patients; closed circles = control subjects. Training produced a significant reduction of ADP levels at all matched submaximal workloads and even at peak exercise. ** = comparison by ANOVA of detraining studies with control subjects; † and †† = comparison of training with detraining studies by ANOVA († $p<0.05$, ** and †† both $p<0.01$).

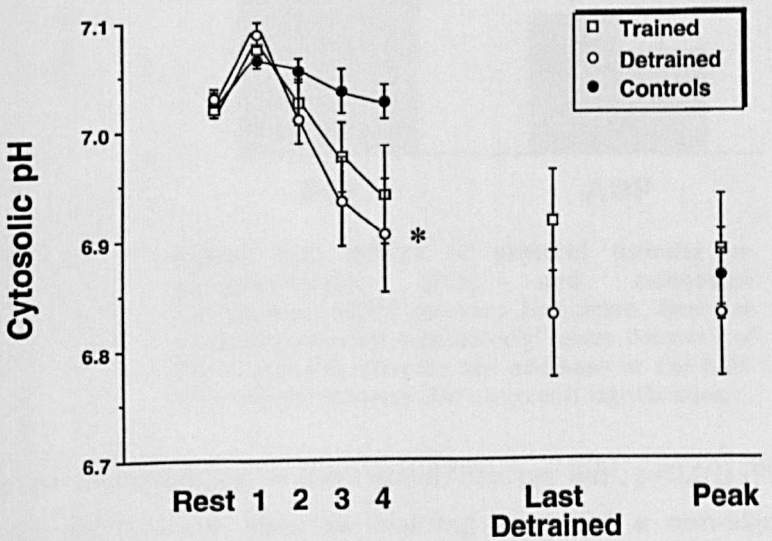

Figure 6.5. Effects of physical training on pH. Rest = resting muscle; 1-4 = the first four spectra (each 64 sec) obtained at 1.5-W workload of plantarflexion exercise; Last detrained = the highest workload achieved in the detraining study; Peak = highest workloads achieved in detraining and training studies; open squares = trained patients; open circles = detrained patients; closed circles = control subjects. Training did not induce any significant difference in pH changes during plantarflexion exercise. * = comparison by ANOVA of detraining studies with control subjects (* $p<0.05$).

4. *pH.* Physical training produced no significant difference in muscle pH throughout plantaflexion at all matched workloads or at peak exercise (Figure 6.5), although a non-significant trend to higher pH levels after training was apparent.

ii. Effects of Physical Training on Recovery Half-Time

1. *Phosphocreatine.* Exercise training produced a significantly shorter recovery half-time of PCr (0.56±0.17 vs 0.89±0.16 min, $p<0.05$), indicating an acceleration in PCr resynthesis during the recovery period (Figure 6.6). After training, the initial rate of PCr resynthesis was faster (16±2 vs 11±2 mmol/liter per min, $p<0.05$) even though the end-exercise [ADP] (which drives PCr resynthesis) was reduced. Thus, the estimated maximal rate of mitochondrial ATP synthesis, calculated from the initial PCr resynthesis rate and end-exercise [ADP], was

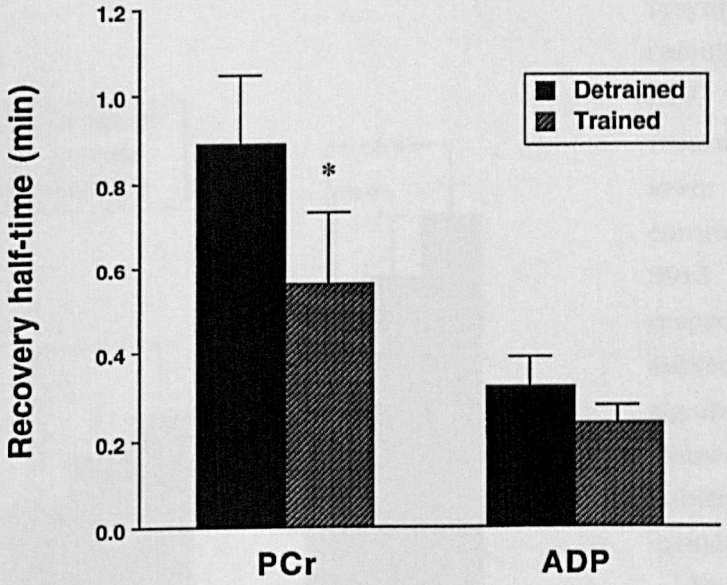

Figure 6.6. Effects of physical training on phosphocreatine (PCr) and adenosine diphosphate (ADP) recovery half-times. Exercise training produced significantly faster recovery of PCr (* $p<0.05$) whereas the decrease in the half-time of ADP recovery did not reach significance.

increased after training (33±6 vs 20±3 mmol/liter per min, $p<0.01$) (Figure 6.7).

2. *Adenosine diphosphate.* Exercise training produced a non-significant trend towards shorter recovery half-time of ADP (0.24±0.04 vs 0.32±0.07 min, $p=0.1$), indicating an acceleration in restoration of ADP to normal during the recovery period (Figure 6.6).

iii. Comparisons Between Patients and Control Subjects

Compared with control subjects, untrained patients with CHF had a significantly increased [ADP] ($p<0.005$), increased PCr depletion ($p<0.001$) and increased acidification ($p<0.04$) during exercise at 1.5-W, which was the highest workload that all control subjects and patients with CHF managed to perform (Figures 6.3 to 6.5).

There was no significant difference in pH and cytosolic free [ADP] between control subjects and patients with CHF after training either at rest or at all four spectra throughout exercise at 1.5-W. Phosphocreatine depletion, however, was significantly less in the control subjects ($p<0.05$) (Figures 6.3 to 6.5).

There was no significant difference between trained or untrained patients and control subjects in recovery half-time of PCr (0.61±0.08 min in control subjects) or ADP (0.29±0.04 min in control subjects). However, in detrained patients both the initial rate of PCr resynthesis and the calculated maximal rate of ATP synthesis during recovery were significantly lower (both $p<0.003$) than in control subjects (20±2 and 39±3 mmol/litre per min, respectively, in control subjects); there was no significant difference between trained patients and control subjects with respect to these measurements (Figure 6.7).

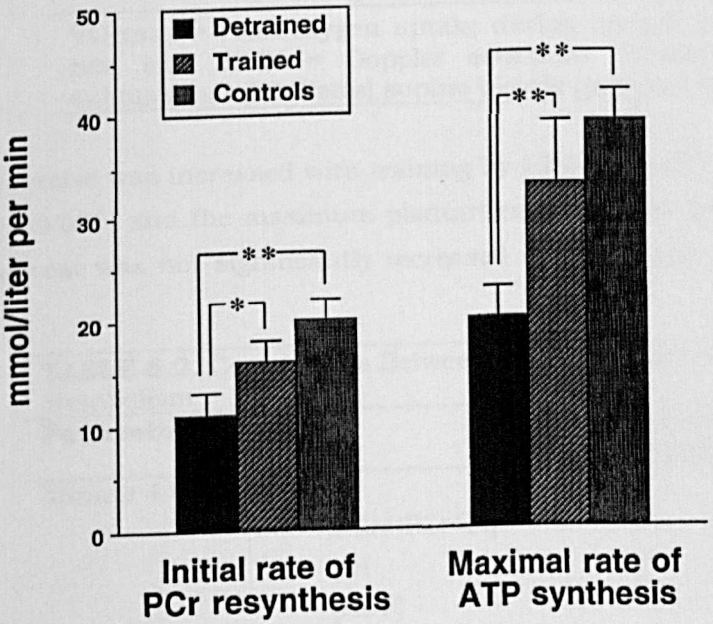

Figure 6.7. Effects of physical training on initial rate of phosphocreatine (PCr) resynthesis and maximal rate of mitochondrial ATP synthesis. The initial rate of PCr resynthesis was faster (* $p<0.05$) and the maximal rate of mitochondrial ATP synthesis was increased (** $p<0.01$) after training. In detrained subjects both measurements were also significantly lower (both ** $p<0.003$) than in control subjects; no significant difference, however, was found between trained and control subjects with respect to these parameters.

Thus, physical training in patients with CHF partially corrected skeletal muscle metabolic abnormalities. However, the plantarflexion exercise time of 9.4±0.8 min after training was still shorter ($p<0.001$) than the exercise time of 19.7±1.2 min achieved by control subjects (Figure 6.2).

iv. Correlations Between Cardiac Performance and Skeletal Muscle Metabolism

Physical training induced an improvement in functional capacity and exercise tolerance in patients CHF (Table 6.1). Maximal oxygen consumption measured during upright bicycle

TABLE 6.1. Bicycle and Leg Plantarflexion Exercise Performance; Effects of Physical Training

Parameter	Detraining	Training	Significance
VO_2max (ml/kg/min)	12.1±1.2	14.1±1.5	p<0.004
Bicycle exercise duration (min)	12.7±1.7	15.8±2.1	p<0.002
Plantarflexion exercise tolerance (min)	7.4±0.7	9.4±0.8	p<0.001
Cardiac output rest (L/min)	5.0±0.4	5.7±0.5	NS
Cardiac output 50-W (L/min)	6.1±0.6	6.9±0.7	p<0.05

VO_2max = Peak oxygen uptake during upright bicycle exercise; Cardiac output rest and 50-W = Doppler estimated cardiac output at rest and during submaximal (50 Watts) supine bicycle exercise; NS = not significant.

exercise was increased with training by 13% (p<0.004), the bicycle exercise duration by 19% (p<0.002) and the maximum plantarflexion exercise time by 27% (p<0.002). Cardiac output at rest was not significantly increased with training, whereas cardiac output at 50-W was

TABLE 6.2. Correlations Between Cardiac Performance and Skeletal Muscle Metabolism

Parameter		VO2max (ml/kg/min)	Cardiac output at 50-W (L/min)
Minute 4 of exercise			
	PCr/(PCr+Pi)	0.5	0.5
	pH	0.4	0.5
	[ADP]	0.1	0.1
Recovery half-time			
	PCr	0.3	0.1
	ADP	0.3	0.6

The Table shows the correlation coefficient describing the relationship between the fractional improvement (achieved by training) in each pair of measurements. None of the correlations reaches significance.

significantly increased with training by 22% (p<0.05). There was no significant correlation, however, between improvement in bicycle or plantarflexion exercise performance and training-induced changes in skeletal muscle metabolism (Table 6.2), nor was there a significant correlation between improvements in estimated maximal rate of PCr resynthesis and the PCr/(PCr+Pi) at minute 4 of exercise (r=0.5, p=NS).

E. Discussion

i. Phosphorus-31 Magnetic Resonance Spectroscopy Abnormalities

This study demonstrates that in patients with moderate to severe ischaemic CHF an exercise training programme at home improves skeletal muscle bioenergetics. This improvement in high energy phosphate metabolism in muscle is in addition to the training-induced improvements in exercise performance, haemodynamics, ventilation, and autonomic function that has been shown in patients with CHF (Coats *et al.*, 1990 and 1992a).

The reduction in PCr utilisation and the smaller rise in [ADP] during exercise suggest an increased capacity for oxidative ATP synthesis after physical training as a result of an increase in either mitochondrial content or functional capacity of the existing mitochondria; it has been recently shown (Dudley *et al.*, 1987) that the relationship between work output and free ADP during steady-state muscle contraction was shifted by varying the mitochondrial content of muscle. The acceleration of PCr resynthesis during recovery is consistent with this view, and the increase in the inferred maximal rate of ATP synthesis suggests an approximately 50% improvement. It has been also recently demonstrated that the volume density of mitochondria in muscle and the surface density of mitochondrial cristae are reduced in CHF (Drexler *et al.*, 1992) and that regular physical training increases mitochondrial volume density (Hambrecht *et al.*, 1995); beneficial changes in volume density of cytochrome *c* oxidase-positive mitochondria in leg muscle were correlated with changes in oxygen uptake at ventilatory threshold, indicating that an increased oxidative capacity of the mitochondria may account for the improved functional work capacity after exercise training (Hambrecht *et al.*, 1995). Recent investigations from the same group (Hambrecht *et al.*, 1999) reported a reduction in mitochondrial creatine kinase, a key enzyme responsible for rapid energy transfer from mitochondrium to cytosol, thus partially explaining the delayed resynthesis of PCr during exercise in skeletal muscle of patients with CHF. Decreased mitochondrial creatine kinase and exercise capacity were inversely correlated with increased expression of inducible nitric oxide synthase (iNOS) in the skeletal muscle of patients with CHF, possibly indicating that NO produced by iNOS attenuates mitochondrial energy transfer (Riede *et al.*, 1998; Hambrecht *et al.*, 1999). Given the exeperimental evidence that NO can also attenuate contractile performance of skeletal muscle and can mediate muscle wasting, exercise training might exert its beneficial effects by decreacing local production of NO through reduction of iNOS gene expression in trained CHF patients (Kobzik *et al.*, 1994).

Changes in pH during exercise are a balance betwen the effects of lactic acid production and buffering, and an important component of buffering during exercise is the consumption of protons by the net hydrolysis of PCr (Kemp *et al.*, 1993b). Training produces no significant change in the pH response during exercise compared with detraining in patients with CHF. This could be because both PCr depletion [reflected in changes in PCr(PCr+Pi)

ratio, Figure 6.3] and lactic acid production are reduced after training. However, despite similar PCr depletion in the trained and control subjects with CHF (Figure 6.3), trained subjects show significant acidosis during exercise (Figure 6.5). This observation suggests that the patients with CHF may continue to have increased lactate production even after training.

ii. Possible Biochemical Mechanisms

Further insight into the biochemical substrate of our training-induced bioenergetic changes is provided by measurements of muscle enzymatic activity in our previously reported experimental model (Brunotte *et al.*, 1995). Rats with heart failure responded to a physical training programme by increasing levels of the mitochondrial oxidative enzyme citrate synthase and the mitochondrial-cytoplasmic enzyme glutamate pyruvate aminotransferase. Training is known to induce an increase in the level of glutamate pyruvate aminotransferase, which katalyses the generation of alanine and ketoglutarate from pyruvate and glutamate, thus reducing the formation of lactic acid during exercise (and therefore acidification of the muscle during exercise), providing at the same time more oxaloacetate for the first step of Krebs cycle (Mole *et al.*, 1973; Holloszy, 1976). Reduced glutamate pyruvate aminotransferase activity in CHF might be another biochemical mechanism explaining the improvement in skeletal muscle abnormalities and exercise capacity in our patients with CHF.

Apart from muscle conditioning there are other possible explanations of the training induced improvement in skeletal muscle oxidative capacity. Lipkin *et al.* (1988) and Miyagi *et al.* (1991) reported evidence consistent with reduced skeletal muscle mass and strength in CHF. Thus, in CHF each fibre might be subjected to an increased load, resulting in a greater change in PCr/(PCr+Pi) ratio and muscle pH. The MRS changes during exercise seen in our patients after training, therefore, could have resulted from performing the same work with more muscle (induced by physical training). That this is not the only mechanism has been shown in a study (Minotti *et al.*, 1990b) in which localised skeletal muscle training produced beneficial MRS responses at submaximal workloads without any associated change in muscle mass. Moreover, in our recent experimental study (Brunotte *et al.*, 1995), no significant difference in muscle mass was found in rats with congestive heart failure randomly allocated to either training or non-training compared with values in sham-operated rats despite the significant training-induced improvement in skeletal muscle metabolism assessed with ^{31}P MRS and enzymatic assays. Also, there is evidence (Mancini *et al.*, 1992b) that atrophy contributes only modestly to reduced exercise capacity and altered muscle metabolism in CHF.

It is a particular advantage of measurements of PCr recovery kinetics that they are independent of muscle mass (Mancini *et al.*, 1992b). We and others (Mancini *et al.*, 1992b) have shown that PCr resynthesis is impaired in CHF and the present data show that this is improved by training. A novel feature of the present analysis is the estimation of the maximal rate of mitochondrial ATP synthesis by using the known relation between PCr

resynthesis and [ADP] (Kemp *et al.*, 1993a). This represents the inferred rate of ATP synthesis at saturating ADP concentrations and is (in accordance with standard biochemical usage) the mitochondrial effective maximal rate of mitochondrial ATP synthesis (Q_{max}). This takes into account the relation between ATP synthesis and [ADP] (Kemp *et al.*, 1993a) and addresses questions of mitochondrial capacity and control more directly than does the recovery half-time.

iii. Possible Neural and Vascular Mechanisms

Wilson *et al.* (1984b and 1986) reported impairment in blood flow during exercise in patients with heart failure. Although inadequate blood flow could produce the MRS changes observed in our patients, there is strong evidence from blood flow measurements (Wiener *et al.*, 1986; Arnolda *et al.*, 1990; Massie *et al.*, 1987a) and studies during ischaemic exercise (Massie *et al.*, 1988) that these metabolic events are, to an extent, independent of changes in limb blood flow. The training-induced biochemical improvements in our study could be explained by a reduced peripheral resistance with concomitant increase in peak blood flow to the exercising leg and markedly reduced arterial and venous lactate levels that have been reported by Sullivan *et al.* (1988a). The same investigators, however, provide evidence that after training the lactate production was delayed during exercise, independent of leg blood flow and O_2 delivery at submaximal exercise. These human data confirm the finding in previous experimental studies (Musch *et al.*, 1986) where no difference in blood flow was found between trained and sedentary rats with myocardial infarction. Minotti *et al.* (1990 a&b) in their localised forearm exercise training study in patients with CHF showed improvement in muscle energetics independently of limb blood flow or central cardiovascular response. Also, the same investigators (Minotti *et al.*, 1991b) more recently reported that reduced muscle endurance is independent of exercise blood flow and suggested that structural or biochemical changes, or both, in muscle may contribute to exercise intolerance in CHF. However, despite these findings, redistribution of blood flow within the leg to perfuse the exercising muscles more effectively cannot be excluded as a cause of the decreased exercise tolerance (Mackie *et al.*, 1983).

A contribution of peripheral neural adjustments to the metabolic changes after training also cannot be excluded. These may be related to either alterations in recruitment of motoneurones with endurance training or increased sensitivity of β_2 muscle metabolic receptors compatible with the reduced sympathetic activity we have observed with training (Coats *et al.*, 1992a).

iv. Conclusions

To what extent these muscle changes influence the exercise capacity of patients remains to be determined. The lack of correlation between the training-induced improvement in exercise performance and indices of skeletal muscle metabolism (seen in Table 6.2) indicates that other peripheral factors may be limiting exercise in these patients. Once skeletal

muscle changes are corrected, peripheral vascular, autonomic or pulmonary factors may intervene to restrict the improvement in exercise tolerance. Training is also specific to the training task and because we have trained our patients on a bicycle and tested with plantarflexion exercise, the changes may be disparate (Rube *et al.*, 1990). This view is also supported by the finding that in patients with CHF training almost completely corrected the skeletal muscle metabolic abnormalities observed in comparison with findings in age-matched control subjects but only very partially corrected the reduced plantarflexion exercise tolerance. When one limitation to exercise is specifically improved, another seems to come in to limit overall exercise performance. Further prospective trials early in the evolution of heart failure would be necessary to show whether some of the secondary changes could be ameliorated by avoidance of physical deconditioning.

Chapter VII

Effects of Physical Training on Autonomic Function in Chronic Heart Failure

A. Abstract

Objectives and Background. Physical training improves exercise performance, ventilation, skeletal muscle metabolism and symptoms in patients with CHF. Physical deconditioning may cause or perpetuate some of the secondary changes seen in CHF. These include excessive neurohormonal vasoconstrictor activity and alterations in autonomic control mechanisms, which may exacerbate symptoms and effort intolerance. There has been no prospective, controlled study of the effects of physical training on autonomic function in CHF.

Methods and Results. In a controlled crossover trial of 8 weeks of exercise training, 25 men with stable moderate to severe CHF (age: 61.6±1.6 years; left ventricular ejection fraction: 21.6±2%) increased exercise performance when assessed by either upright bicycle exercise duration (from 14.0±0.9 to 16.6±1 min, $p<0.001$) or peak oxygen uptake (from 13.6±0.8 to 15.9±0.9 ml/kg/min, $p<0.001$). Sympatho-vagal balance was improved by physical training when assessed by three methods : 1) Heart rate variability in the time domain expressed by standard deviation of normal morphology R-R intervals (SDRR, +31.6 %, $p<0.01$); 2) Heart rate variability in the frequency domain by using autoregressive power spectral analysis of the resting ECG divided into low-frequency (LF, -19.4%, $p<0.01$) and high-frequency (HF, +85%, $p<0.01$) components; and 3) Whole-body radiolabeled NA spillover (-22.6%, $p<0.05$). These measurements all showed a significant shift away from sympathetic towards increased vagal activity after training.

Conclusions. Carefully selected patients with moderate to severe CHF can achieve significant, worthwhile improvements in autonomic control with physical training. This raises the possibility that the autonomic imbalance associated with CHF may in part be due to chronic physical deconditioning and may at least be partially reversible by physical exercise.

B. Introduction

The clinical syndrome of CHF is associated with abnormalities in autonomic control of the circulation. Chronic activation of the adrenergic system, decreased parasympathetic activity and impaired arterial baroreflex sensitivity have all been described as part of the syndrome (Ferguson *et al.*, 1984, Leimbach *et al.*, 1986; Cohn *et al.*, 1988; Eckberg *et al.*, 1997). The endogenous release of vasoconstrictor neurohormones is due to activation of the sympathetic nervous, renin-angiotensin and arginine vasopressin systems (Francis, 1985), and although acting initially as a compensatory response, appears to play a deleterious role in the development of CHF, increasing the loading conditions of the failing ventricle and predisposing to occurence of complex ventricular arrhythmias (Packer *et al.*, 1986). Reduced HRV and baroreflex sensitivity and increased plasma NA levels have all been shown to be independent predictors of increased mortality in CHF (Cohn *et al.*, 1984; Kleiger *et al.*, 1987; La Rovere *et al.*, 1988) and therefore these abnormalities may be prognostically important.

Therapeutic interventions, which may at least in part restore this impaired neurohormonal balance, produce haemodynamic and clinical benefits in patients with CHF. Recent reports have suggested a restoration of the baroreflex gain and HRV after pharmacological treatment (Marin-Neto *et al.*, 1991) or transplantation (Smith *et al.*, 1989). Angiotensin-converting enzyme inhibitors and ß-blockers, when given in addition to conventional therapy have been recently shown to significantly reduce mortality and hospitalisations for congestive heart failure (The SOLVD Investigators, 1991; MERIT-HF and CIBIS-II studies, 1999). Angiotensin-converting enzyme inhibitors have been suggested to increase vagal activity in CHF (Osterziel *et al.*, 1988), whereas digoxin has been demonstrated to produce early profound and sustained reduction in sympathetic nerve activity in patients with CHF (Ferguson *et al.*, 1989). Non-pharmacological approaches to reducing sympathetic activity and/or increasing vagal tone and baroreceptor sensitivity could also be of considerable importance, given the association between sympathetic hyperactivity and/or reduced HRV and increased mortality (Cohn *et al.*, 1984; Kleiger *et al.*, 1987).

There are many similarities between the abnormalities associated with CHF and those seen in physical deconditioning. In both conditions there is exercise intolerance, sympathetic hyperactivation (Cooksey *et al.*, 1978; Leimbach *et al.*, 1986; Saul *et al.*, 1988), increased resting heart rate, reduced HRV (Pagani *et al.*, 1988; Smith *et al.*, 1989), wasted skeletal muscle (Holloszy, 1976; Buller *et al.*, 1991) and depleted skeletal muscle oxidative enzymes (Holloszy, 1976; Green *et al.*, 1980; Sullivan *et al.*, 1990). Physical deconditioning in CHF may, therefore, contribute to some of the secondary abnormalities seen in CHF such as changes in the neurohormonal axis, skeletal muscle metabolism and the autonomic control of the cardiovascular system (Francis, 1985; Massie *et al.*, 1987b). Recent investigations have established that physical training has beneficial effects in compensated heart failure on exercise tolerance, central and peripheral haemodynamics, ventilation, symptomatic status and metabolic responses (Conn *et al.*, 1982; Sullivan *et al.*, 1988a and

1989a; Coats *et al.*, 1990). Also experimental and human studies in our laboratory suggest that an exercise training programme can produce a substantial correction of the impaired oxidative capacity of skeletal muscle in CHF (Adamopoulos *et al.*, 1993; Brunotte *et al.*, 1995), consistent with increased mitochondrial content and/or improved respiratory function of the existing mitochondria. This finding seems to be in keeping with other recent investigations (Sullivan *et al.*, 1988a), which have shown that physical training induces markedly reduced arterial and venous lactate levels during exercise independent of improvements in leg blood flow.

Physical training has been shown to enhance HRV and baroreflex gain in subjects with normal left ventricular function (Pagani *et al.*, 1988) and to modify the sympaho-vagal control of HRV toward a persistent enhancement in parasympathetic tone when performed early (8 weeks) after a myocardial infarction (Malfatto *et al.*, 1996). Moreover, the combination of rehabilitation and ß-blockers after acute myocardial infarction induced a more favourable sympatho-vagal balance, accelerating the recovery of a normal autonomic profile (Malfatto *et al.*, 1998). We designed a controlled crossover trial comparing home-based exercise training with activity restriction to look at the effects of exercise training in stable CHF. In addition to conventional haemodynamic, exercise and symptom measures, we used three methods of assessing sympatho-vagal balance (HRV in the time domain, power spectral analysis of HRV and NA spillover) to evaluate critically whether short-term exercise training programme could modify the abnormalities of autonomic function seen in CHF. The only prospective, controlled study of the effects of physical training on some haemodynamic and autonomic parameters in the first 17 patients with CHF has been reported by our group (Coats *et al.*, 1992a).

C. Methods

i. Study Design

The study, which was approved by the Central Oxford Research Ethics Committee, was a randomised crossover comparison of 8 weeks of exercise training against 8 weeks of restricted activity (rest). Two to 3 baseline laboratory visits were used to familiarise the patients to the exercise tests and other laboratory procedures. The physician conducting the tests was unaware of the training status of the patients. Habituation to test procedures was avoided by the patients not attending the hospital during the 8 weeks of either phase of the study.

ii. Study Population

All subjects gave informed consent for this trial. Inclusion criteria were stable CHF (NYHA class II-III) of at least 3 months' duration; ischaemic aetiology (21 patients, as evidenced by documented myocardial infarction and/or coronary arteriography and coronary bypass surgery) or idiopathic dilated cardiomyopathy (4 patients); stable sinus rhythm; limitation of

exercise by symptoms of dyspnoea or fatigue only and ability to reach a respiratory exchange ratio of at least unity; absence of Holter monitoring evidence of sustained ventricular tachycardia or other serious arrhythmias; absence of symptomatic angina or ECG evidence of ischaemia limiting exercise. Radionuclide ventriculography was performed at baseline, but the results did not constitute part of the entry criteria. This test was not repeated during the study (it was not felt justified to ask the subjects to undergo 3 scans for this trial) and did not constitute one of the tests of efficacy.

iii. Training Programme

The patients were instructed on the use of a training bicycle (Tunturi Professional Ergometer, Tunturi, Finland) that they kept for their use at home for 8 weeks. They were instructed to exercise at 50 rpm for 20 min 5 days per week and to keep the resistance setting of the bicycle so that their continuously monitored heart rate (Micro Sports Lab Computer, Triadcolour, London) was kept in the range of 60-80% of their previously determined maximum heart rate. Compliance was assessed as the percentage of expected bicycle wheel revolutions achieved over the training period.

iv. Control Rest Phase (Detraining)

The patients were asked to avoid exercise above their normal prestudy routine and specifically to avoid exercise that would lead to feelings of dyspnoea or exhaustion. No formal compliance assessment was made. The patients did not have the exercise bicycle in their houses during this phase of the trial.

v. Exercise Testing

At each visit, after overnight fasting and before administration of medication, the patients performed an incremental (4-min, 25-W stages) upright bicycle exercise test to exhaustion, with 1-min average measurements of respiratory gas exchange. Inspiratory flow (Harvard Instruments Dry Gas Meter) and expiratory oxygen and carbon dioxide concentrations (Servomex 570A and PA404 meters, Servomex, Crowborough, Sussex, England) were measured, and oxygen consumption and carbon dioxide production were calculated by standard formulas. The gas meters were calibrated against gases of known concentrations before each test.

vi. ECG Recordings

1. *Heart Rate*

During exercise. Heart rate was recorded from the electrocardiogram at the end of each stage of the bicycle exercise test and was derived from the average of 20 beats. A three-lead ECG was recorded throughout exercise.

Twenty four-hour Holter monitoring. Twenty-four hour ECG monitoring was performed with a 2-channel (modified V_1 and V_5 leads) recorder (Oxford Medilog II, Oxford Med. Instruments). Qualitative and quantitative ECG analysis was performed using a

computerised non-triggered template system consisting of a Z80A preprocessor and DEC-LSI master, which was developed and validated in our laboratory (Rossi *et al.*, 1983). Analysis of the recordings was performed blind to the current training status.

Tapes were analysed to obtain the mean and standard deviation of all R-R intervals that had normal morphology and cycle lengths within 80% and 120% of the preceding cycle duration. Results are presented separately for the whole 24 hours and separately for daytime (14-20 hours) and nighttime (0-6 hours). These 6-hour periods were chosen as being unaffected by the trip to the hospital and most likely to represent daytime and sleeping states. They are not, however, full summaries of waking and sleeping heart rate behaviour.

2. *Heart Rate Variability*

Data acquisition. During supine rest in a quiet, darkened room, 640 consecutive heartbeats were recorded on a Store 4 Racal-Thermionic FM tape recorder (Southampton, UK) at a tape speed of 15/16 inches/sec. Lead V_5 was used for this recording. These recordings were used to perform standard deviation of laboratoty R-R intervals (time domain) and power spectral analysis of variations in R-R interval (frequency domain). No special attempts were made to control or alter the patient's respiratory pattern during this recording, but special care was taken that the patient was relaxed, stable and undisturbed.

Signal acquisition and power spectral analysis of R-R interval variability. The tape recorded resting data, from the laboratory recording period, were digitized off-line by a 12 bit analog-to-digital converter (NB-MIO-16 board, National Instruments, Austin, Tx) at a sampling rate of 500 samples per sec. The converter was connected to a MacIntosh IIcx computer (Apple Inc., Coupertino, Ca) equipped with 5-Mb RAM memory and a 60-Mb hard disk. A "C" language program identified all the QRS complexes in each sequence and located the peak of each R wave. From these data, the R-R intervals were obtained. For each sequence, 256 heart beats were analysed. Trends were removed from each sequence by subtraction of that same sequence after a 124-lag window smoothing procedure, following a previously described algorithm (Press *et al.*, 1986). Premature beats were interactively identified and corrected by linear interpolation with the previous and following beats.

Power spectral analysis of the R-R interval signal was performed by an autoregressive model on 256 consecutive heart beats (Kay and Marple, 1981). Model coefficients were evaluated according to a modification of the Burg algorithm (Ulrych and Bishop, 1975; Pagani *et al.*, 1986) and model order was assessed by Akaike criteria (Zetterberg, 1969; Pagani *et al.*, 1986); a model order between 9 and 13 was found to be adequate in most cases. Spectral components were obtained by a decomposition method previously described (Zetterberg, 1969; Kay and Marple, 1981), which was also used to measure the area below each spectral peak (Pomeranz *et al.*, 1985). We expressed the variation in Hertz rather than in cycles per beat, assuming that the R-R interval changes were small with respect to the mean R-R interval and considering the sampling time equal to the mean R-R interval. The power spectrum shows two separate peaks: the higher frequency (HF) peak is related to respiration; the low-frequency (LF) peak appears unrelated to any respiratory event (Kay and

Marple, 1981; Akselrod *et al.*, 1985; Pomeranz *et al.*, 1985). Occasionally, the LF peak is further subdivided into a LF and a very low-frequency peak (VLF), but this was not done in this study because the VLF peak can be difficult to differentiate from LF noise unless the recording period is very prolonged.

Power spectral analysis is a relatively new technique and some explanation of its capabilities and limitations may be helpful. Spectral analysis is a mathematical technique in which variations in heart rate are analysed in the frequency domain. In other words, this analysis can tell us how much of the variability of R-R intervals occurs in a regular rhythmic fashion and how much of it occurs at different frequencies. From experiments on parasympathetic and ß-blockade in dogs (Akselrod *et al.*, 1985) and humans (Pomeranz *et al.*, 1985) it appears that the HF rhythm is entrained by the respiratory frequency and, apart from a small residual (probably mechanical) respiratory component seen after cardiac transplantation (Bernardi *et al.*, 1989), is carried almost entirely by vagal activity. The LF rhythm is mediated jointly by the vagus and the sympathetics but predominantly by the latter (Kay and Marple, 1981; Akselrod *et al.*, 1985; Pomeranz *et al.*, 1985).

We evaluated the power of the harmonic components in the range between 0.03 and 0.14 Hz (LF component) and those in the range 0.18 and 0.40 Hz (HF component). To simplify comparison of spectra, we considered the relative percentage of each spectral component compared with the total oscillatory power and expressed it as normalised units (nu). In addition, we evaluated the absolute value of the HF area as an expression of respiratory sinus arrhythmia (RSA).

The relative amount of HF component and the absolute amount of RSA have been considered as indices of parasympathetic activity, whereas the relative amount of the LF component and the LF/HF ratio have been considered as indices of sympathetic activity (Pomeranz *et al.*, 1985; Pagani *et al.*, 1986). The LF/HF ratio is more recently viewed as reflecting sympatho-vagal balance (Malliani *et al.*, 1991). It must be stressed, however, that power spectral measurements are not strictly quantitative, because it is impossible to assess absolute measurements of oscillatory power in different frequency ranges without taking into account differences in HRV. It has not been shown that power within a certain frequency band can be used to quantify differences in sympathetic of parasympathetic tone between individuals. Mostly, the ratio between LF and HF power is used, and this ratio does not lend itself to simple quantitative analysis. No gold standard measure of autonomic balance exists. The power spectral technique gives qualitative and semiquantitative information, which, when combined with other autonomic measures, can help describe autonomic nervous control of the intact circulation.

vii. Noradrenaline Kinetics

Noradrenaline kinetics were measured according to the techniques of Esler *et al.* (1979) in 17 subjects. 1-[2,5,6-^{3}H]NA (New England Nuclear, Boston Massachusetts) was given intravenously as a bolus [12 microCi (0.44 MBq)] followed by constant infusion [0.7 microCi (0.026 MBq)/min^{-1}m^{-2}] for up to 60 min. Arterial and venous blood samples were collected

at rest and at submaximal (50-W) supine bicycle exercise in both training and detraining phases of the study. The blood samples were analysed for plasma NA using high-performance liquid chromatography and NA plasma clearance was then measured as has been previously described (McCance and Forfar, 1989a). The normal range of plasma NA in our laboratory is 120-300 pg/ml. Plasma [^{3}H]NA was measured by liquid scintillation counting of alumina extracts with correction for recovery of a non-radioactive internal standard (dihydroxybenzylamine). Noradrenaline plasma clearance and whole-body NA spillover were measured according to the following relations, which hold under steady-state conditions

$$\text{Whole-body NA clearance} = \frac{\text{Infusion rate (dpm/min)}}{\text{Plasma [}^3\text{H]NA (dpm/ml)}}$$

$$\text{Whole-body NA spillover} = \frac{\text{Infusion rate (dpm/min)}}{\text{Specific radioactivity of plasma NA (dpm/pg)}}$$

where dpm is disintegrations per minute of tritiated NA.

viii. Statistical Analysis

The trial was analysed according to the recommendations of Hills and Armitage (1979) for crossover trials and analysed for the presence of period or carry-over effects, none of which were found. Analysis of variance followed by Student's t test was used for normally distributed parameters and Wilcoxon signed-rank test was used for non-normally distributed parametrs. Significance at the 5% level or lower is reported and corrected where appropriate for multiple comparison testing by the Scheffe procedure. Data are presented as mean±SEM.

D. Results

i. Effects of Physical Training on Exercise Tolerance

Compared with the rest (detraining) phase, physical training significantly improved exercise capacity by 19.5% (upright bicycle exercise time from 14.0±0.9 to 16.6±1 min, p<0.001) and

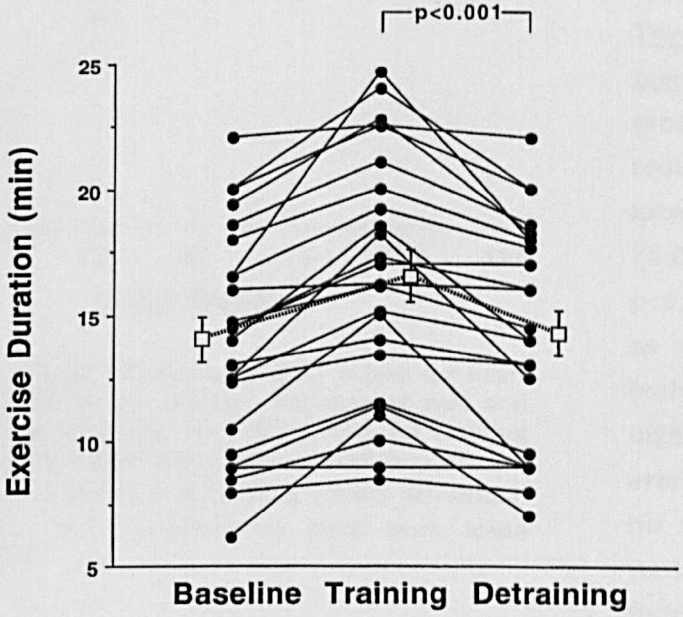

Figure 7.1. Effects of physical training on exercise tolerance estimated by the exercise duration in minutes during upright bicycle exercise. Graph of individual patient upright exercise durations for baseline, training and detraining periods. *** p<0.001 for comparison between training and detraining periods, n=24.

increased peak achieved oxygen consumption by 17.1% (from 13.6±0.8 to 15.9±0.9 ml/kg/min, p<0.01). Individual patient data on the change in exercise time are shown in Figure 7.1. There was no significant difference between the baseline and rest periods.

ii. Effects of Physical Training on Autonomic Balance

1. *ECG Recordings*

During exercise. Upright bicycle exercise was performed after training with lower heart rates at comparable work loads (Table 7.1). After training, there were significant reductions in submaximal heart rate at 25-W and 50-W exercise with no change in peak heart rate (Figure 7.2). Resting heart rate was reduced non-significantly from 83.9±3.2 to 80.9±4 beats per min (bpm) (p=0.08), 25-W heart rate from 103.8±2.8 to 96.5±2.9 bpm (p<0.05), 50-W heart rate from 112.8±3.9 to 104.7±4.3 bpm (p<0.05), and peak heart rate unchanged (135.2±4.4 to 133.1±5.6 bpm, p=NS). The submaximal workload (50-W) rate-pressure

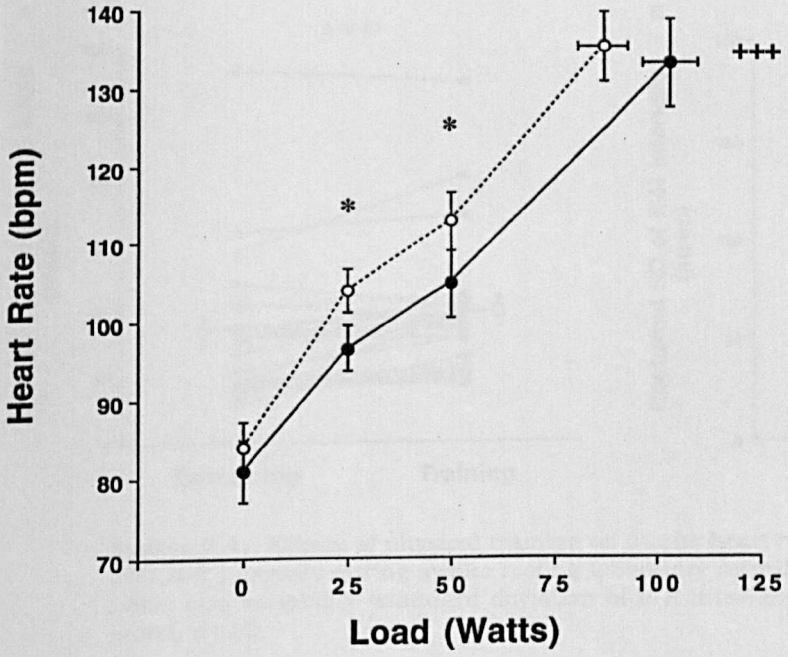

Figure 7.2. Graph shows mean±SEM values for heart rate (bpm = beats per minute) response at rest and different levels of upright bicycle exercise for training (closed circles) and detraining (open circles) periods. * $p<0.05$ for comparison of training versus detraining; +++ $p<0.001$ for comparison of peak work loads achieved, n=24.

product was also significantly reduced by training (-11.2%, $p<0.01$), indicating a more efficient exercise performance with respect to myocardial oxygen requirement.

Twenty four-hour Holter monitoring. Training produced significant reductions in 24-hour mean heart rate (from 76.0±2.9 to 74±2.6 bpm, $p<0.005$, Figure 7.3A), as well as separately for both daytime and nighttime heart rate averages (Figure 7.3B). No serious or complex ventricular arrhythmias were detected on any of the recordings but there was a significant excess of ventricular ectopic beats during the training phase (rest phase, 29.4±8.6 hour^{-1}; training phase, 60.7±24.5 hour^{-1}, $p<0.05$), which may have been related to the slower heart rate unmasking ventricular automaticity. There was no significant change in the rate of early premature ventricular beats.

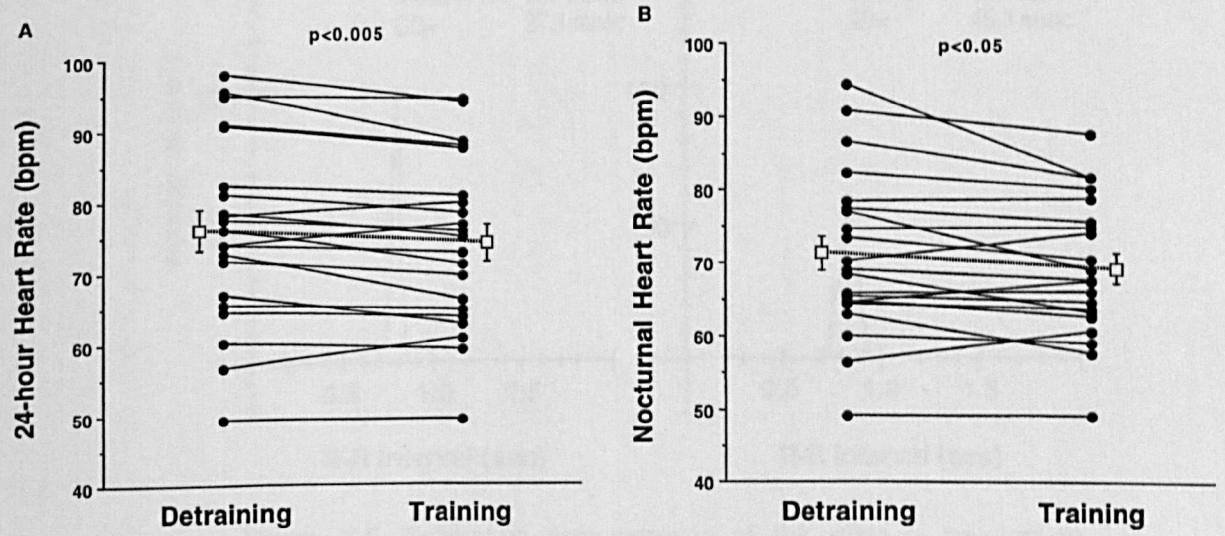

Figure 7.3. Effects of physical training on 24-hour heart rate (left panel, n=21) and nocturnal (0-6 AM) heart rate (right panel, n=21); bpm = beats per min.

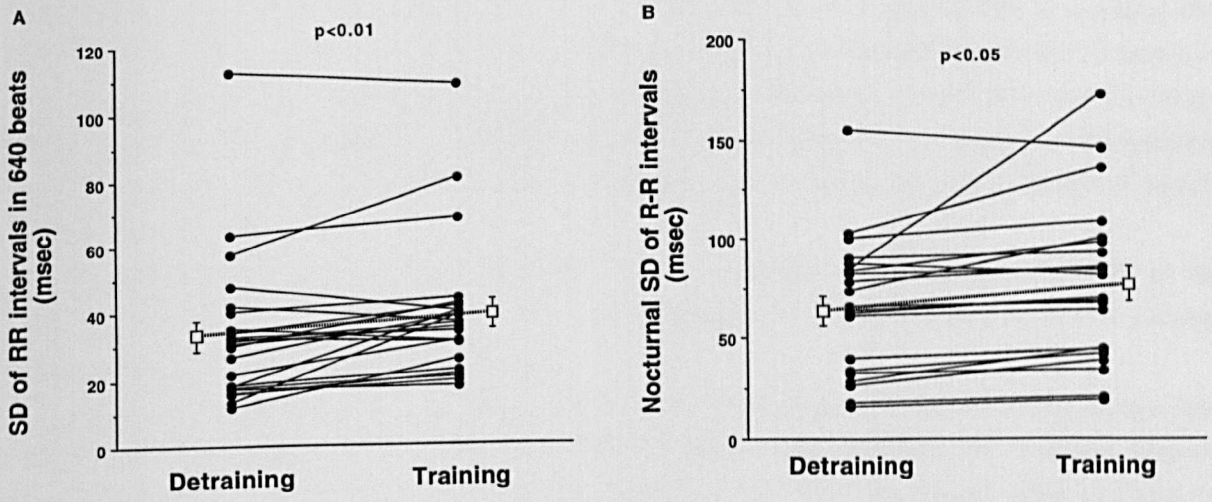

Figure 7.4. Effects of physical training on awake heart rate variability (standard deviation of 640 R-R intervals during awake resting laboratory recording, left panel, n=23) and nocturnal heart rate variability (standard deviation of R-R intervals during sleep from 0 to 6 AM, right panel, n=22).

Physical training significantly increased the 24-hour HRV expressed by the standard deviation of R-R intervals. Thus, the standard deviation of all normal morphology R-R intervals increased by 14.7% (from 96.5±7 to 109.4±8.2 msec, $p<0.05$). As shown for individual subjects in Figure 7.4B nocturnal HRV also increased by 19% (from 65.9±7.5 to 76.9±8.9 msec, $p<0.05$), whereas the daytime HRV was not significantly different (79.8±6.2 versus 88.8±8.4 msec, p=NS), possibly because of the effects of different levels of physical activity between the two phases of the trial.

Resting ECG recording in the laboratory. During supine rest in a quiet, darkened room, 640 concecutive heart beats were recorded as described in "Methods". Only normal

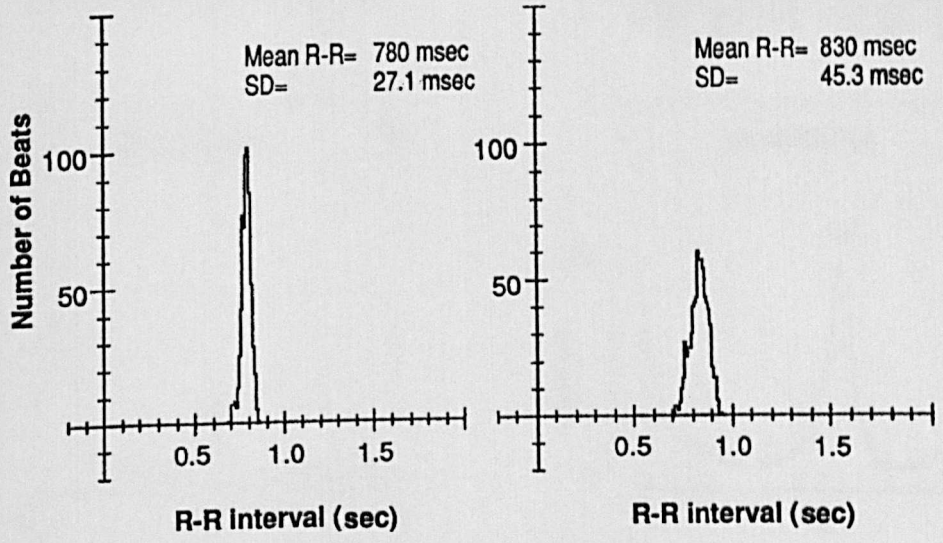

Figure 7.5. Schematic demonstration of the effect of training on resting heart rate variability expressed as standard deviation (SD) of 640 consecutive R-R intervals in the laboratory in a single subject after detraining (left panel) and after training (right panel).

morphology complexes with R-R intervals betwen 80% and 120% of the preceding R-R interval were included in the calculation of HRV to exclude an effect of increased ectopics on the measure of HRV. Resting (awake) heart rate was significantly lower after training (from 73.2±2.9 to 71.3±2.7 msec, $p<0.05$) and resting HRV (indicated by the standard deviation of all 640 concecutive beats) significantly increased with training by 31% (33.2±4.6 versus 39.1±4.4 msec, $p<0.01$).

An individual example of the effects of physical training on resting awake HRV in the time domain is shown by use of an R-R interval frequency histogram in Figure 7.5. Overall patient results are shown in Figure 7.4A.

Power Spectral Analysis. Training effect on the 256-beat R-R interval tachogram and power spectral display is depicted in Figure 7.6 for a single subject. The relative amount (expressed in normalised units) of the LF component of HRV decreased after training by 19.4% (from 62.3±4.4 to 50.5±5; $n=22$, $p<0.01$, Figure 7.7A), as well as the LF/HF ratio by 27.6% (from 4.4±1 to 2.3±0.5, $p<0.05$). Both indices have been shown to reflect changes in sympathetic nervous activity. Three patients of 25 had too many ventricular ectopics to enable power spectral analysis of these recordings. In the other 22, a period of ECG recording with few or no ectopics was chosen for analysis and when no completely ectopic-free period of 256 beats could be found, ectopic beats were deleted and the two R-R intervals affected by this editing were corrected by linear interpolation with the previous and following R-R intervals (see "Methods").

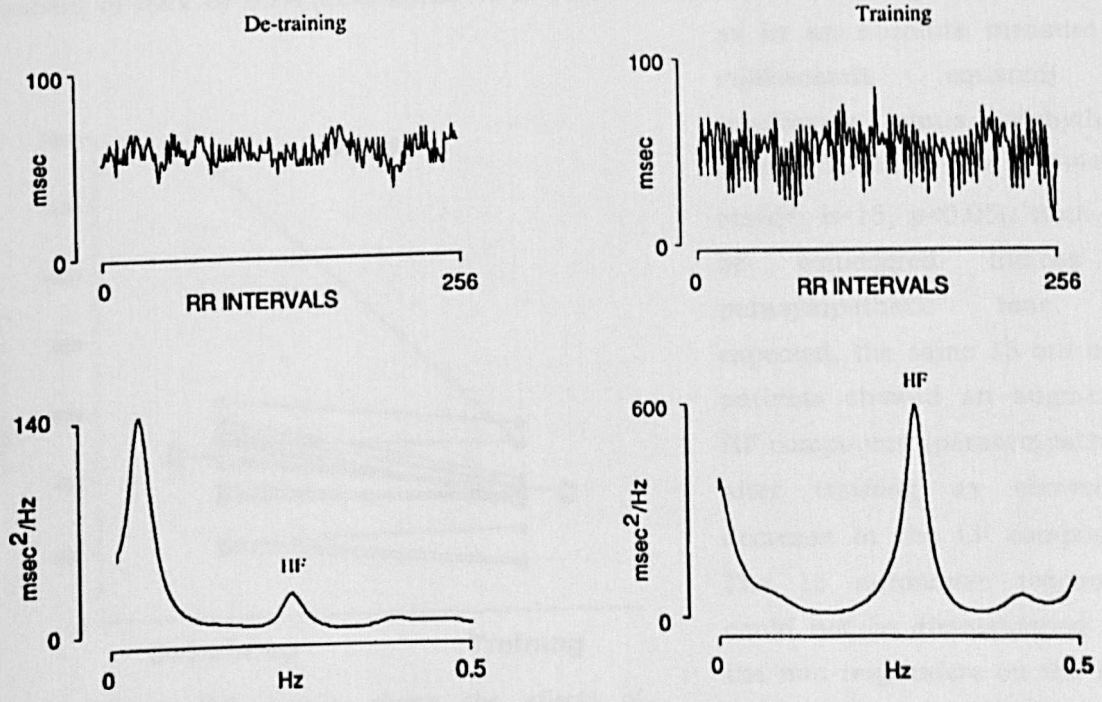

Figure 7.6. Tracings show effect of physical training on the R-R interval tachogram (display of individual R-R intervals) for 256 consecutive beats (upper panels) and on the low- (0.08 Hz, LF) and high-frequency (0.25 Hz, HF) spectral components after power spectral analysis of R-R variability (lower panels) in an individual patient. Note the higher variability after training and its predominant high-frequency pattern.

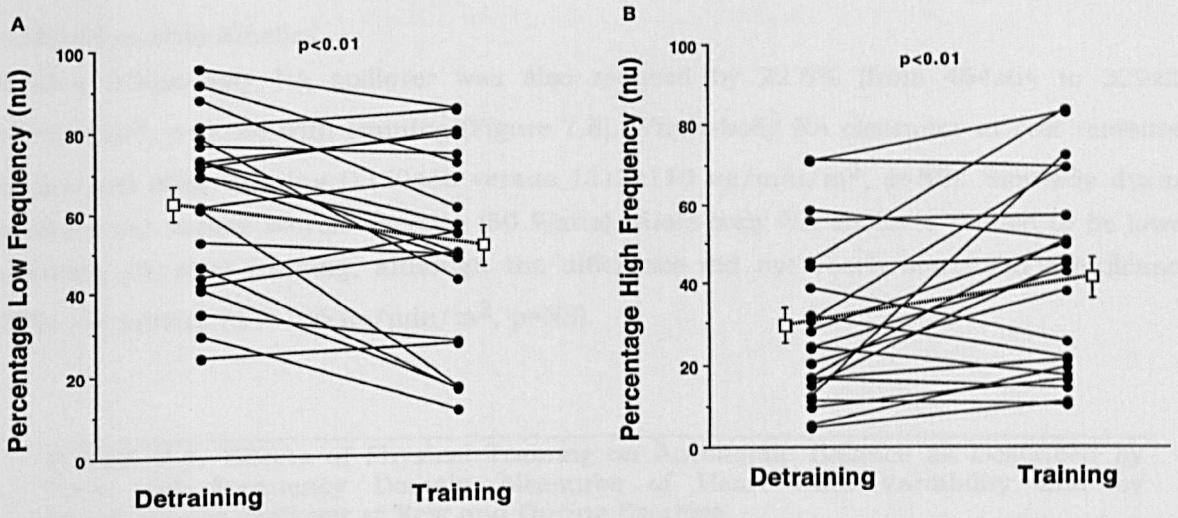

Figure 7.7. Effects of physical training on low-frequency (left panel, n=22) and high-frequency (right panel, n=22) oscillatory components derived from power spectral analysis of heart rate variability; nu = percentage of each spectral component over the total oscillatory power.

In 15 of 22 patients, there was considerable reduction in the LF measure of sympathetic drive after training, whereas the remaining 7 showed no change or a slight increase (Figure 7.7A). The greater degree of variability in this response compared with mean 24-hour heart rate or HRV may reflect a greater degree of inherent variability in this measurement, or, alternatively, a poorer relation to fitness effects.

There was a considerable increase in the relative amount (in normalised units) of the HF component of HRV by 85% (from 29.6±4.3 to 41.6±5.1; n=22, $p<0.01$, Figure 7.7B), as well as in an absolute measure (in milliseconds squared) of respiratory sinus arrhythmia (from 193±146 to 344±201 $msec^2$; n=15, $p<0.05$). Both can be considered indices of parasympathetic tone. As expected, the same 15 out of 22 patients showed an augmented HF component (parasympathetic) after training as showed a decrease in the LF component. The 15 autonomic responders could not be distinguished from the non-responders on the basis of disease severity, drug usage or history of coronary surgery.

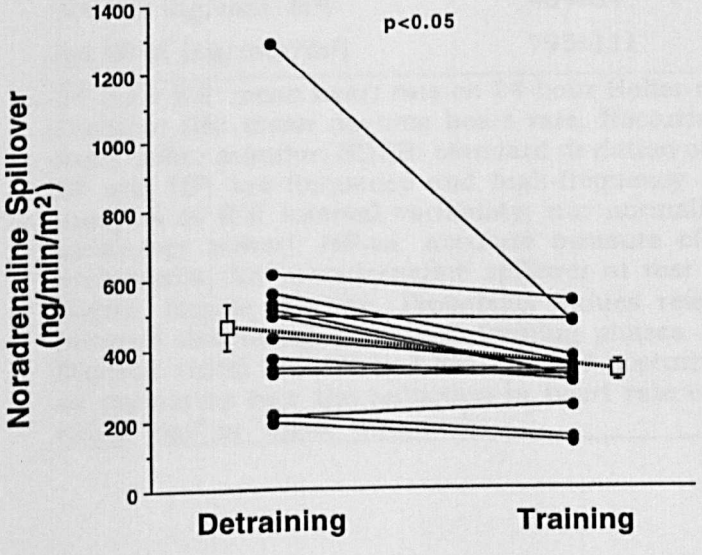

Figure 7.8. Graph shows the effects of physical training on noradrenaline spillover at rest; n=16.

2. *Noradrenaline Kinetics*

Resting whole-body NA spillover was also reduced by 22.6% (from 464±64 to 329±26 ng/min/m^2, $p<0.05$) with training (Figure 7.8). Whole-body NA clearance at rest remained unchanged after training (1159±58 versus 1313±110 ng/min/m^2, p=NS). Similarly during submaximal supine bicycle exercise (50 Watts) whole-body NA spillover tended to be lower following physical training, although the difference did not reach statistical significance (795±111 versus 721±106 ng/min/m^2, p=NS).

TABLE 7.1. Effects of Physical Training on Autonomic Balance as Described by Time and Frequency Domain Measures of Heart Rate Variability and by Noradrenaline Spillover at Rest and During Exercise

Measures	**Detraining**	**Training**	**p-value**
24-hour HR (bpm)	76.0±2.9	74.0±2.6	<0.005
Daytime* HR (bpm)	81.2±3.2	79.0±3.3	<0.05
Nocturnal* HR (bpm)	71.2±2.5	69.3±2	<0.05
Submaximal HR (bpm)	112.8±3.9	104.7±4.3	<0.05
SDRR 24 hours (msec)	96.5±7.0	109.4±8.2	<0.05
SDRR daytime (msec)	79.8±6.2	88.8±8.4	NS
SDRR nocturnal (msec)	65.9±7.5	76.9±8.9	<0.05
LF (nu)	62.3±4.4	50.5±5	<0.01
HF (nu)	29.6±4.3	41.6±5.1	<0.01
HF-sa ($msec^2$)	193±146	344±201	<0.05
Low/High-Frequency ratio	4.4±1.1	2.3±0.5	<0.05
NA rest (ng/min/m^2)	464±64	329±26	<0.05
NA 50-W (ng/min/m^2)	795±111	721±106	NS

24-hour HR: mean heart rate on 24-hour Holter monitoring; bpm: beats per minute; Daytime HR: mean daytime heart rate; Nocturnal HR: nighttime mean heart rate from Holter monitor; SDRR: standard deviation of normal morphology R-R intervals. LF and HF: low-frequency and high-frequency components of the power spectral analysis of R-R interval variability; nu: normalised units (percentage of the total oscillatory power); HF-sa: absolute measure of high-frequency respiratory sinus arrhythmia; NA: noradrenaline spillover at rest and during 50 Watts submaximal supine bicycle exercise. Probability values refer to Student's *t* test comparison between detraining (rest) and training phases. NS: not statistically significant. * Daytime refers to 2 PM to 8 PM only and nocturnal refers to midnight to 6 AM only, so explaining how the reduction in heart rate over 24 hours can be less than for either "day" or "night" separately.

iii. Correlations Between Measurements of Autonomic Balance and Improved Exercise Performance

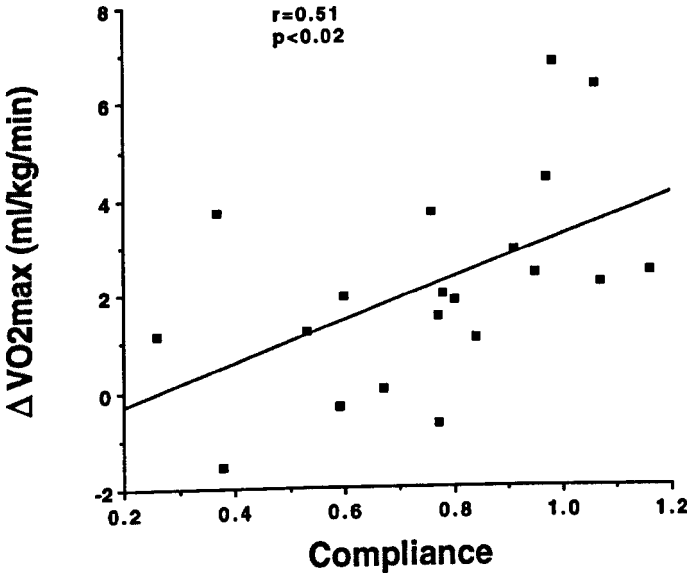

Figure 7.9. Graph shows relation between percentage increment in exercise tolerance against compliance to the training programme; n=20.

None of the baseline characteristics significantly predicted improvement in either exercise capacity or autonomic control of the cardiovascular system; in particular there was no evidence for the more severely affected patients with lower initial left ventricular ejection fractions or lower peak oxygen uptakes having a different response to training. There was, however, a strong correlation between improvement and the independently measured compliance to the programme ($r=0.51$, $p<0.02$); this relation is shown in Figure 7.9. There was no significant correlation between the severity of heart failure (measured by either ejection fraction or peak oxygen uptake) and compliance to the programme.

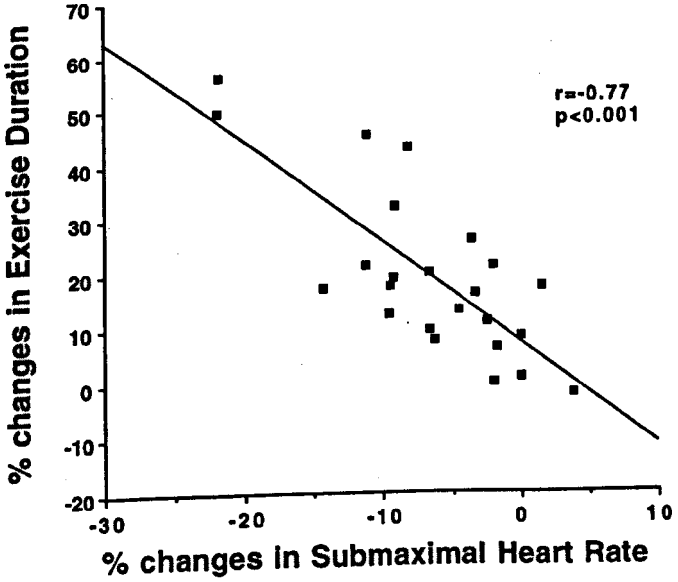

Figure 7.10. Correlation between the percentage (%) reduction in submaximal heart rate (during upright bicycle exercise) and the percentage increase in upright bicycle exercise time after training; n=24.

We investigated whether other patient characteristics were related to the extent of training-induced changes in the measures of autonomic function. There was no significant effect on these changes (either absolute or percentage change) of the site of prior myocardial infarction, the history of coronary bypass grafting, the duration of heart failure symptoms or the taking of or duration of any particular cardiovascular medication. There were only 3 subjects

taking digoxin, only 1 on a calcium antagonist and only 3 not taking an ACE inhibitor; therefore, these nubmers were too small to determine whether these medications significantly affected the training response.

We determined which training-induced change was best correlated with the improvement in exercise performance as measured by the percentage increase in exercise duration. The percentage reduction in submaximal exercise heart rate was the best correlate of improvement in exercise performance ($r=-0.77$, $p<0.001$, Figure 7.10). In addition, the percentage increase in HRV correlated strongly with the improvement in exercise tolerance ($r=0.71$, $p<0.001$, Figure 7.11 left panel). The correlation between the increase in supine exercise tolerance and the percentage reduction in NA spillover was also strong ($r=-072$, $p<0.01$).

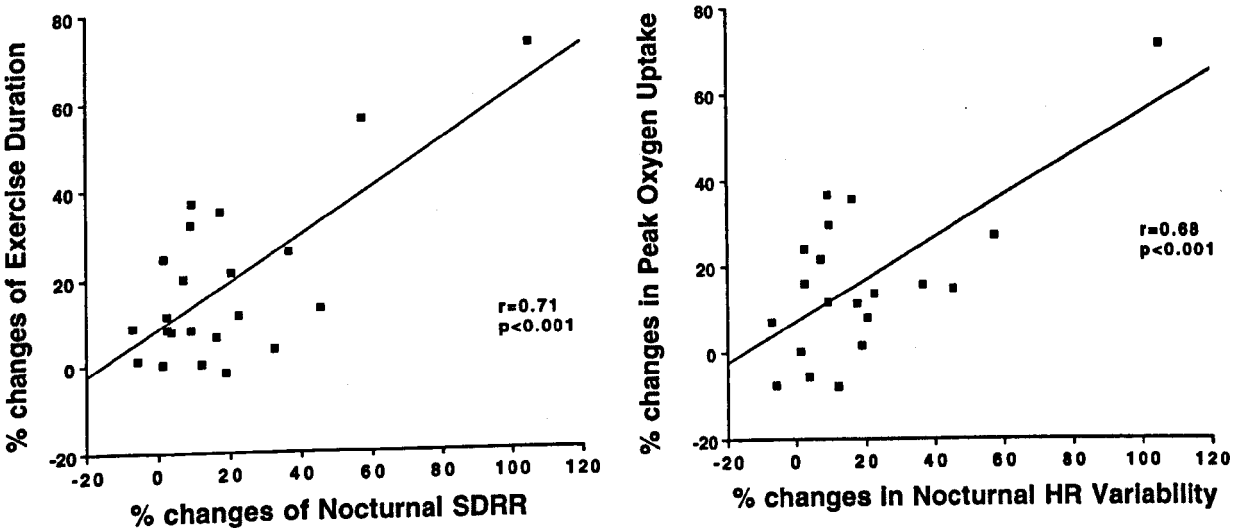

Figure 7.11. Correlation between the percentage (%) increase in nocturnal standard deviation (SD) of R-R intervals and the percentage increase in upright bicycle exercise time after training (left panel, n=22) or the percentage increase in peak oxygen uptake after training (right panel, n=20).

Using the increase in peak oxygen uptake as the dependent variable instead of exercise duration, very similar correlations were found. The percentage increase in nocturnal HRV and the percentage reduction in submaximal exercise heart rate were the strongest correlates of improvement ($r=0.68$, $p<0.001$, Figure 7.11 right panel and $r=-0.60$, $p<0.01$ respectively). Other changes were not significant at the 1% level.

At baseline, the different methods of estimating autonomic balance correlated poorly and non-significantly with each other, with the exception of the expected inverse correlation between LF and HF components of HRV ($r=-0.92$, $p<0.001$). All methods, however, showed an improvement in sympatho-vagal balance after training and, as described in the previous paragraph, the changes in each method produced by training were well correlated with improved exercise tolerance.

E. Discussion

In CHF, acute pharmacologically or transplant-induced improvements in haemodynamics are not associated with immediate improvements in exercise performance, because the increased exercise tolerance often takes several weeks to develop (Sinoway *et al.*, 1988; Drexler *et al.*, 1989). This may be due to the need for increased activity to reverse some of the secondary abnormalities of CHF (sympathetic activation, reduced HRV, wasted skeletal muscle and impaired skeletal muscle oxidative capacity), which may themselves have actually become the limiting factors to exercise. This study gives some insight into whether exercise training alone can partially reverse the autonomic features of deconditioning seen in CHF and thereby improve symptoms and exercise performance.

Previous investigators have shown a training-induced increase in exercise tolerance and reduction in blood pressure and heart rate in subjects with normal left ventricular function and minor left ventricular dysfunction (Letac *et al.*, 1977; Lee *et al.*, 1979; Blomqvist 1983). Sinus bradycardia of endurance athletes is a well recognised phenomenon. Several theories have been recruited to explain the underlying mechanism including enhancement of vagal tone (Frick *et al.*, 1967), reduced ß-receptor activity (Ekblom *et al.*, 1973), resetting of arterial baroreceptors (Mitchell and Wildenthal, 1974) and primary adjustment of the intrinsic heart rate (Lewis *et al.*, 1980). However, it has been recently demonstrated that cardiac vagal adaptation plays an important role in regulating sinus node activity in highly trained endurance athletes (Dixon *et al.*, 1992). Decreased resting sympathetic tone and increased parasympathetic activity are part of the training response in normal subjects (Blomqvist, 1983, Seals and Chase, 1989). We have previously shown that training improves exercise performance in moderate to severe heart failure patients (Coats *et al.*, 1990).

In our study, physical training in heart failure patients produced significant, and perhaps important, reductions in 24-hour heart rate, day and night heart rate, reductions in exercise heart rate and rate-pressure product, markedly increased HRV in both waking and sleep states and significant reduction in NA spillover. These findings may reflect beneficial changes in the autonomic balance and/or baroreflex-cardiopulmonary receptor activity, in agreement with the increased vagal tone found after endurance exercise training in healthy sedentary men (Somers, 1986a).

Arterial and cardiopulmonary baroreceptor dysfunction, associated with the loss of atrial compliance and the normal structure of the atrial receptors and the reduction of the signals from ventricular afferent epi-myocardial fibres (Muers and Sleight, 1972; Hirch *et al.*, 1987), may precede the development of overt heart failure in dogs; reversal of experimental heart failure is associated with normalisation of baroreceptor function (Higgins *et al.*, 1972). Baroreflex insensitivity, which may play a central role in the activation of vasoconstrictor systems in heart failure, and impaired parasympathetic to sympathetic balance are both associated with reduced HRV, an index of reduced parasympathetic activity (Kleiger *et al.*, 1987). Physical training may enhance baroreceptor responsiveness (Somers, 1986a; Seals

and Chase, 1989), either by improving cardiac performance (reducing filling pressure and increasing contractility and thus restoring the afferent signals from the cardio-pulmonary receptors) or by reducing central inhibition of the baroreflex loop, perhaps by reduced angiotensin II. In addition, there may be an improvement in left ventricular pumping capacity, perhaps resulting from a true positive inotropic effect of training as seen in animals (Crews and Aldinger, 1967).

It is possible that additional factors are operative in explaining the beneficial effects of physical training in patients with CHF. Muscle wasting and underperfusion and tissue acidosis, which accompanies deconditioning, may increase sympathetic activation. Training by virtue of its beneficial effects on exercising muscle (Adamopoulos *et al.*, 1993) and peripheral circulation (Sullivan *et al.*, 1988a) may improve skeletal muscle metaboreceptor responses, which contribute to the regulation of the overall sympathetic activity (Sterns *et al.*, 1991), and thus reverse favourably the sympatho-vagal imbalance seen in CHF. A recent report by our group (Piepoli *et al.*, 1996) suggests that the muscle "ergoreflex" role has a larger effect on the circulatory responses to exercise (including activation of the sympathetic vasoconstrictor drive) in CHF than in control subjects and that training may reduce this exaggerated ergoreflex activity.

A number of studies have been conducted (Akselrod *et al.*, 1981; Lombardi *et al.*, 1987; Sands *et al.*, 1989) using power spectral analysis of the HRV as a non-invasive means of assessment of the "tonic" autonomic input to the heart. The quantitative analysis of the low- and high-frequency heart rate fluctuations has, however, been little used to assess the autonomic balance in a controlled intervention trial in heart failure. Thus, increase in the HF component during both sedentary and active periods and attenuation of the LF/HF ratio during the day were recently observed in patients with CHF underwent 3 months of physical training (Kiilavuori *et al.*, 1995). Also, a training-induced enhancement in sympathetic modulation of peripheral vessels has been demonstrated in patients with CHF by using the power of the oscillations of blood pressure (Radaelli *et al.*, 1996). In our study, a reduced sympathetic activity after training, suggested by the finding of a decreased LF component and LF/HF ratio, was confirmed by the reduction of the whole-body NA spillover without associated changes in plasma NA clearance. Even this method is not a direct measure of sympathetic activity, as it cannot differentiate between different causes for the measured increase in the rate at which NA spills over from the nerve terminals in CHF. This could be due to increased presynaptic release of NA, decreased uptake of NA by uptake$_1$ or a combination of mechanisms. We have no information in this study on the mechanisms of the apparently reduced sympathetic activity. In parallel, vagal tone was augmented as indicated by the increase of the relative and absolute oscillatory power of the HF component, the increase in HRV and the reduction in heart rate both during exercise and on 24-hour ECG monitoring.

Although this study cannot tell us the precise mechanisms of the training-induced improvements in exercise capacity, but as the changes in heart rate and NA spillover would allow the same work load to be achieved at a lower sympathetic drive, heart rate and

myocardial oxygen requirement it is then plausible that reserve exercise capacity could be enhanced.

Although the total number of ventricular ectopics in a 24-hour period increased after training, the frequency of R-on-T extrasystoles or more serious arrhythmias did not. Training-induced bradycardia may unmask automaticity of the ventricles by lowering heart rate and allow more benign "escape" ventricular premature beats because of slow heart rate. Although overnight and resting (awake) HRV both increased with training, daytime HRV did not. This might imply a more effective sympathetic myocardial chronotropic responsiveness during physical activity or greater daily activity during the training phase.

The positive effects of physical rehabilitation in chronic stable heart failure were recently confirmed by the European Heart Failure Training Group in a large cohort of heart failure patients (European Heart Failure Training Group, 1998). A significant training effect in exercise tolerance (13% increase in peak oxygen consumption and 17% increase in exercise time duration) was associated with improved autonomic indices (HRV in the time and frequency domain, resting catecholamines and hormones), without significant side-effects and with similar benefits in a variety of conditions and different hospital settings. Thus, there was no indication of any differences in the effects of physical training on the exercise capacity and autonomic function when the patients were subdivided according to age, disease severity and duration, history of coronary artery bypass grafting or pharmacological treatment.

Physical training may improve not only physical fitness but also prognosis, as has been suggested in patients with ischaemic heart disease without heart failure (O'Connor *et al.*, 1989), perhaps by partially correcting abnormalities associated with increased mortality (Szlachcic *et al.*, 1985; Myers *et al.*, 1986; Bigger *et al.*, 1992b), such as reducing NA spillover and improving exercise tolerance and HRV measures in the time and frequency domain. Reduction in the adrenergic and increase in the vagal tone have been recently proposed as the main mechanism underlying the favourable outcome in the only, so far, study translating a sustained improvement in functional capacity and quality of life into a better clinical outcome after long-term moderate exercise training (Belardinelli *et al.*, 1999).

The various individual methods for assessing sympatho-vagal balance correlated non-significantly between each other. Despite the improvement in all individual measures of autonomic control after training, there was again no correlation between the training-induced changes in those parameters. On the contrary, there were strong correlations between the enhancement of HRV and improved exercise performance after training ($r=0.71$, $p<0.001$), between the training induced reduction in submaximal heart rate and improved exercise capacity ($r=-0.77$, $p<0.001$), and between reduction in NA spillover and increase in peak oxygen uptake ($r=-0.58$, $p<0.02$). These correlations might be reflecting the close relationship of increased fitness to reduced heart rate and NA spillover and increased HRV. Thus, there may be a beneficial feedback between improvement in cardiovascular function and shifts in autonomic balance from sympathetic to vagal predominance.

The lack of inter-method correlation even after an intervention, such as physical training, which improved all the autonomic function parameters, suggests that in CHF individual measures of autonomic balance may reflect different aspects of circulatory control and a comprehensive description of the autonomic status necessitates more than one method (see detailed description of inter-method comparisons in Chapter IV).

i. Comparison with Other Therapeutic Approaches

Previous reports have shown that the reduced HRV in heart failure is partially corrected by cardiac transplantation. Our values of HRV in the resting phase are similar to those described by Smith *et al.* (1989) before transplantation. The change in HRV, produced by training, seen in our study is of similar magnitude to the increased variability described after transplantation. Increased physical activity and training effects after transplantation may explain a large part of the improved HRV described by Smith and colleagues and may also explain the delay in improvement seen after cardiac transplantation (Sinoway *et al.*, 1988). Similar delays are described after ACE inhibitor treatment and may similarly reflect the need to undo some of the effects of physical deconditioning (Drexler *et al.*, 1989).

ii. Clinical Implications

These studies demonstrate significant improvements in the autonomic control of heart rate and HRV as well as in NA kinetics in CHF after physical training. Prolonged inactivity leading to deconditioning may, thus, be partly responsible for the abnormalities of autonomic control of heart rate described in CHF. The endogenous release of vasoconstrictor neurohormones may eventually play a deleterious role in the development of the clinical syndrome of CHF, by increasing the loading conditions of the failing heart and favouring the development of complex arrhythmias. Exercise training programmes in heart failure may, therefore, not only improve exercise performance but may also improve prognosis because of reversing, at least partially, adverse prognostic features such as increased NA spillover and reduced HRV.

iii. Limitations of the Study

Our study population was largely consisted of patients with CHF of ischaemic origin. No patients with limiting angina were recruited into this trial but with the known benefits of exercise training on angina thresholds (Todd and Ballantyne, 1990) and collateral development (Eckstein, 1957), it is likely that training would be of benefit in the patients with mixed heart failure and angina. It may seem surprising that patients with an ischaemic aetiology did not have ECG evidence of myocardial ischaemia but some had had bypass grafting and all were highly selected so that reversible myocardial ischaemia was not a cause of exercise limitation. In addition, no patients with serious ventricular arrhythmias (e.g., ventricular tachycardia) were recruited; hence, it may be argued that these patients are for both these reasons not truly representative of a general CHF population. These

results remain valid for this specific group and similar studies in different groups of heart failure patients would clearly be worthwhile.

The methods for assessing autonomic function have not been rigidly validated in CHF because no gold standard exists that can be used in intact humans. Differences in ß-receptor responsiveness will affect nerve traffic measures (e.g., NA spillover) differently to end-organ response measures (e.g., heart rate and power spectral measures). No data exist on the effect of physical training on ß-receptor function in CHF. The wide range of autonomic functional measures used here, all showing a similar trend, go some way to overcoming the lack of a truly representative and quantitative measure of sympathetic and vagal control of the circulation.

At what stage heart failure patients should be advised to train cannot be definitively answered in this study. Training is beneficial after the early healing phase of myocardial infarction (Malfatto *et al.*, 1996; Specchia *et al.*, 1996); provided that the patient is stable with no evidence of exercise-induced ventricular arrhythmia and provided that diuretic treatment is optimised, training should be recommended down to the severity of patients in this report. There is growing evidence that skeletal muscle metabolism and autonomic function become abnormal early after an extensive myocardial infarction (Adamopoulos *et al.*, 1999); therefore, further prospective trials early in the evolution of heart failure would be necessary to show whether the secondary changes (autonomic imbalance and skeletal muscle histological and metabolic abnormalities) could be ameliorated by avoidance of physical deconditioning. The intensity of training, should, however, be customised for each patient and only recommended after detailed cardiac and cardiopulmonary evaluation.

Chapter VIII

Circadian Pattern of Heart Rate Variability in Patients with Chronic Heart Failure

Effects of Physical Training

A. Abstract

Objectives. The effects of physical training on the circadian pattern of HRV (recorded over 24 hours in relation to both time and frequency) was assessed in 12 patients with moderate to severe CHF randomised, in a crossover design, to 8 weeks home-based training or detraining (20 min/day x 5 days/week at 70-80% of maximal heart rate) and compared with 12 age-matched normals.

Methods and Results. Physical training significantly improved HRV indices: the mean of all 5-min standard deviations of R-R intervals (SDNN index) increased by 17.6%, the root mean square of the differences of succesive R-R intervals (rMSSD) by 34.9%, the percentage difference between adjacent normal R-R intervals >50 msec (pNN50%) by 112.5%, total power by 58.3%, high-frequency power by 128.5% and low-frequency power by 65%. The circadian pattern of HRV was assessed by calculating low-frequency (LF) and high-frequency (HF) power and their ratio for each hour. Compared with normal subjects, circadian variations in autonomic parameters were maintained in CHF in both training and detraining conditions. Training-induced changes were observed at different time intervals throughout the day: the highest values were at 0100h-0700h (detraining: LF 361±83 $msec^2$, high-frequency 126±47 $msec^2$; training: low-frequency 535±202 $msec^2$, HF 227±115 $msec^2$, $p<0.01$) and the lowest at 1300h-1900h (detraining: LF 91±23 $msec^2$, HF 39±14 $msec^2$; training: LF 154±42 $msec^2$, HF 133±67 $msec^2$, $p<0.05$).

Conclusions. In patients with CHF, exercise training preserves the circadian variations in HRV measures, whilst improving them in both time and frequency domains. These beneficial changes may lessen the predisposition to ventricular arrhythmias and reduce mortality in CHF.

B. Introduction

Time and frequency domain measures of HRV have been recently used to describe and semiquantitatively characterise the sympatho-vagal activity in normal subjects and in patients after myocardial infarction (Kitney and Rompelman, 1980; Pomeranz *et al.*, 1985; Pagani *et al.*, 1986). Recent investigations have also suggested a potential role of HRV measures to predict both death and arrhythmic mechanisms early and late after myocardial infarction (Lombardi *et al.*, 1987; Farrel *et al.*, 1991; Bigger *et al.*, 1992 a&b and 1993).

Circadian variation of acute cardiovascular events has been recently associated with a well-defined diurnal pattern of dynamic changes in sympatho-vagal balance, as inferred from heart rate and arterial pressure variabilities (Furlan *et al.*, 1990). Frequency domain measures of HRV have shown that a sudden rise in sympathetic activity and reduction in vagal tone in the early morning hours indicates a possible link between the increased rate of cardiovascular events and circadian oscillations in sympatho-vagal interaction on heart rate.

The clinical syndrome of CHF is associated with impaired autonomic control in the cardiovascular system. Chronic enhancement of adrenergic activity and a reduction in HRV and baroreflex sensitivity have all been described as part of the syndrome (Eckberg *et al.*, 1971; Leimbach *et al.*, 1986; Ferguson *et al.*, 1992). Recent reports have suggested a restoration of the baroreflex gain and HRV after pharmacological treatment or transplantation (Smith *et al.*, 1989; Marin-Neto *et al.*, 1991). Physical training improves autonomic function as assessed by NA spillover and HRV measures in time and frequency domains and taken from ECG recordings of short duration (Adamopoulos *et al.*, 1992; Coats *et al.*, 1992a). To our knowledge, however, there is little information concerning the circadian fluctuations of sympathetic and vagal regulation of heart rate in patients with CHF. Interpretations of the circadian pattern of sympatho-vagal balance in CHF may lead to the rationalisation of therapeutic approaches in this category of patients by modifying the HRV markers of autonomic control as well as their circadian variations.

In our study we sought a) to describe the circadian pattern of sympatho-vagal balance in patients with CHF using HRV, by analysing 24-hour ECG recordings, b) to investigate how the circadian pattern could be modified by intervention, i.e. physical training, that partially reverses abnormalities in CHF, c) to evaluate the effect of a training programme on the various parameters characterising HRV in the time and frequency domains, some of which have been shown to acquire prognostic significance (Cohn *et al.*, 1984; Kleiger *et al.*, 1987; La Rovere *et al.*, 1988, Ponikowsky *et al.*, 1997a), and on the circadian variations of these parameters and finally d) to compare the training-induced changes in HRV measures and their circadian pattern, with those of a control group of age-matched normal subjects.

C. Methods

i. Study Population

All subjects gave informed consent for this trial, which was approved by the Central Oxford Research Ethics Committee. We studied patients with stable CHF secondary to ischaemic heart disease, without limiting angina or arrhythmias. They were all male, mean age 62.4±2.0 (range 48-75) years. Six were New York Heart Association class II and 6 class III. Radionuclide left ventricular ejection fraction was 18.9±2.2% and peak oxygen uptake (VO_2max) 12.4±1.2 ml/kg/min; three had undergone coronary artery by-pass grafting. All subjects were taking diuretics (median frusemide dose 80 mg); eleven out of 12 were on ACE inhibitors but none was receiving any ß-blocker therapy. Pharmacological treatment was stable for 3 months prior to and for the duration of the study in all subjects. Our control group was consisted of 12 age-matched healthy males, average age 60.2±1.8 (range 46-71) years; none had a history of cardiac or respiratory disease, or was taking any medication. None of the subjects was a smoker.

ii. Study Design

Upon entry to the trial, CHF patients underwent a 2-4week familiarisation and baseline evaluation phase, during which reproducible exercise and autonomic tests were obtained. Subsequently, all patients were randomised to 8 weeks home-based bicycle exercise (training) or avoidance of exercise (detraining) in a crossover design, that has been previously described by us (Coats *et al.*, 1990). For training, patients were lent a cycle ergometer (Tunturi Professional Ergometer, Tunturi, Finland) for use 5 days each week for 8 weeks. They warmed-up with the resistance set at 25-W for 1 min and then increased until the heart rate was 70-80% of their previously determined maximum. Patients used an electrocardiographically based pulse monitor (Micro Sports Lab Computer, Triad-colour, London, UK) to follow their heart rate during exercise. In the detraining period, the exercise bicycle was removed and the patients were instructed to avoid all exercise that induced dyspnoea or fatigue.

iii. Heart Rate Variability

1. *Data Acquisition*

All normal control subjects and patients with CHF (after training and deraining) underwent 24-hour electrocardiographic ambulatory monitoring, using a 3-channel amplitude modulated tape recorder (Marquette 8500). The subjects were studied on non-working days and asked to follow their normal daily non-working activities. Both groups underwent a similar degree of physical activity, as assessed by diaries, and reported to have slept normally during the night of electrocardiographic monitoring.

Two monitoring leads, a modified inferior lead and CM_5 were used. The Marquette Laser Holter System (ST segment analysis and arrhythmia analysis programme, version 5.7 software, Marquette Electronics Inc. Milwaukee, Wisconsin) was used for the tape analysis. Each QRS complex was identified and labelled. R-wave detection was checked by visual inspection in order to avoid the acquisition of artifacts. The frequency histogram of the normal R-R intervals was displayed and electrocardiogram strips of the intervals in both tails of R-R distribution were visually checked. In addition, a histogram of the consecutive R-R ratio was analysed and cycles outside 80-120% of preceding R-R intervals were excluded from further analysis. All tapes were subsequently analysed to measure HRV in the time and frequency domains, using a commercially available and validated program (Marquette HRV program, Maruqette Electronics Inc. Milwaukee, Wisconsin). Electrocardiographic recordings were analysed by two independent observers who were unaware of the clinical data and the current training status.

2. *Data Analysis*

a) In the time domain. The following time domain measures of HRV were evaluated from 24-hour electrocardiographic recordings: the mean of all normal R-R intervals, the standard deviation of all R-R intervals (SDRR), the standard deviation of 5 min mean R-R intervals (SDANN), the mean of all 5-min standard deviation of R-R intervals (SDNN index), the root-mean square of differences of successive R-R intervals (rMSSD), and the percentage of adjacent normal R-R intervals more than 50 msec different (pNN50%).

b) In the frequency domain. Spectral measures were computed by fast Fourier transforming of sequential 2-min windowed segments with application of a Hamming window to the averages for each hour and for the entire 24-hour of the recording period. Spectral plots were used to identify two subsets of the frequency domain: LF, 0.04-0.14 Hz and HF, 0.15-0.40 Hz. Spectral power was quantified in these two frequency band widths and spectral plots were squared to quantify power in the two frequency bands (in $msec^2$). Total oscillatory power between 0.01 and 1 Hz was calculated and the ratio of LF/HF power was also estimated. This ratio represents an established index of sympatho-vagal balance and high values for the ratio indicate predominance of sympathetic nervous activity (Pagani *et al.*, 1986). Among the operators, the difference for HRV measures was 5% and intra-observer variability, as assessed by a second count on 12 tapes, was also <5%.

iv. Statistical Analysis

Statistical analysis was performed according to the recommendations of Hills and Armitage for crossover trials (Hills and Armitage, 1979). Analysis of variance, followed by Student's t-test, was used for normally distributed parameters and Wilcoxon signed-rank test for non-normally distributed parameters. Analysis of variance for repeated measures was performed to compare frequency domain measures of HRV during the 24-hour period of observation among training, detraining and normal control conditions. Significance at the level of 5% or lower is reported and corrected where appropriate for multiple comparison testing by the Scheffe procedure. Data are presented as mean±SEM.

D. Results

i. Circadian Pattern of Heart Rate Variability in the Frequency Domain in Normal Subjects

The HF component of HRV was at its highest during the first hours of the day and corresponded to the hours of sleep (0100h-0700h, 499±109 msec^2); it lowered rather

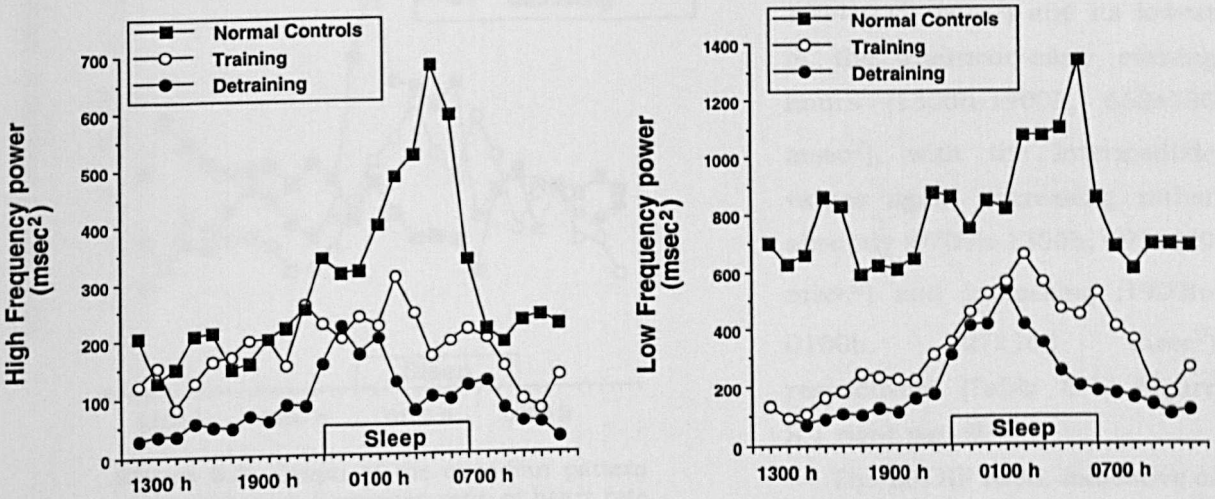

Figure 8.1. Graph of the circadian pattern of high- (left panel) and low- (right panel) frequency components of heart rate variability in control normal subjects (closed squares) and in patients with chronic heart failure after training (open circles) and after detraining (closed circles). Note the higher power of high- and low-frequency during the hours of sleep (2300h-0700h) in all three groups. Note also the higher power of high- and low-frequency in normal subjects compared to patients after both training and detraining and the higher power of high- and low-frequency after training compared to after detraining.

sharply in the morning with enhancement of physical and mental activity (0700h-1300h, 233±39 msec^2), was at its lowest in the afternoon-early evening hours (1300h-1900h,

TABLE 8.1. Circadian Variation in the Frequency Domain Measures of Heart Rate Variability in a Control Group of Normal Subjects

	LF power (msec^2)	**HF power** (msec^2)	**LF/HF** ratio
Time			
7am-1pm	691±129	233±39	4.0±0.6
1pm-7pm	658±185	180±43	4.7±0.5
7pm-1am	727±163	246±72	4.1±0.6
1am-7am	1044±235	499±109*	3.1±0.6

LF = low-frequency; HF = high-frequency.
* $p<0.05$ 1am-7am vs 7am-1pm, 1pm-7pm and 7pm-1am

180±43 msec^2) and progressively increased during the night (1900h-0100h, 246±72 msec^2). The HF component during the first hours of the day (0100h-0700h) was significantly higher compared to all the other periods of the day (ANOVA, $p<0.02$, Table 8.1, Figure 8.1 left panel).

The LF component of HRV followed exactly the same pattern of distribution throughout the day, presenting its highest values during the first hours of the day (0100h-0700h, 1044±235 msec^2) and its lowest in the afternoon-early evening hours (1300h-1900h, 658±185 msec^2), with the intermediate values again decreasing rather abruptly (0700h-1300h, 691±129 msec^2) and increasing (1900h-0100h, 727±163 msec^2) respectively (Table 8.1, Figure 8.1 right panel).

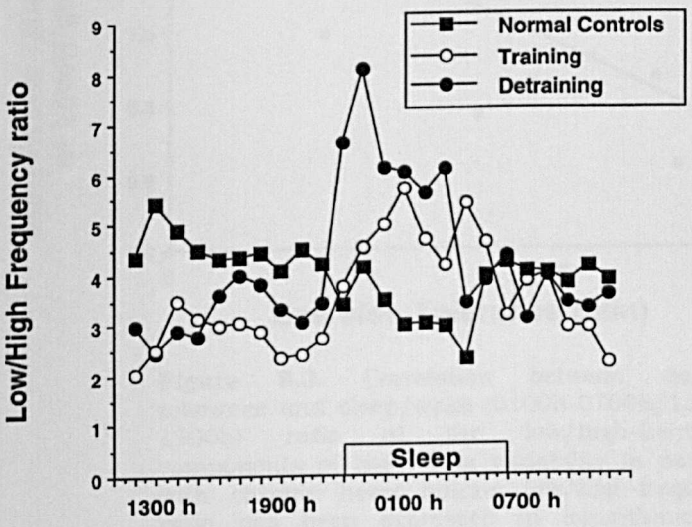

Figure 8.2. Graph of the circadian pattern of the low/high-frequency ratio of heart rate variability in control normal subjects (closed squares) and in patients with chronic heart failure after training (open circles) and after detraining (closed circles). Note the inverse pattern of variation in patients with chronic heart failure after both training and detraining compared to normal subjects, obtaining its highest values during sleep (2300h-0700h) and its lowest during the afternoon-early evening period.

The LF/HF ratio, indicative of the sympathetic activity, was at its lowest while subjects were asleep (0100h-0700h, 3.1±0.6), progressively increasing during the waking period (highest value 1300h-1900h, 4.7±0.5, Table 8.1, Figure 8.2).

ii. Circadian Pattern of Heart Rate Variability in the Frequency Domain in Detrained Patients with Chronic Heart Failure

The circadian variation in the HF and LF components of HRV in patients with CHF followed a pattern similar to that seen in normal subjects, although at a significantly lower power throughout the 24 hours. The LF/HF ratio, however, demonstrated an inverse pattern of variation, obtaining its highest values during sleep and its lowest during the afternoon-early evening period.

The HF component of HRV showed its highest power during the first hours of the day and corresponded to the hours of sleep (0100h-0700h, 126±47 msec^2); it lowered in the morning with enhancement in physical and mental activity (0700h-1300h, 89±35 msec^2), was at its lowest power in the afternoon-early evening hours (1300h-1900h, 39±14 msec^2) and progressively increased during the night (1900h-0100h, 111±54 msec^2, Table 8.2, Figure 8.1 left panel).

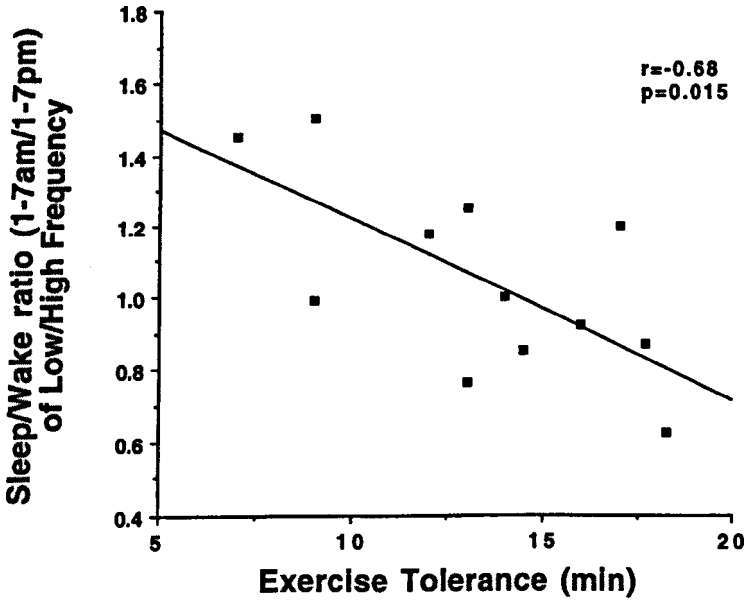

Figure 8.3. Correlation between exercise tolerance and sleep/wake (0100h-0700h/1300h-1900h) ratio of the low/high-frequency components of heart rate variability in patients with chronic heart failure; low/high-frequency ratio has been expressed in logarithmic (ln) values.

The LF component of HRV followed the same pattern of distribution as the HF component throughout the day, presenting its highest power during the first hours of the day (0100h-0700h, 361±83 msec^2), decreasing rather sharply in the morning (0700h-1300h, 159±43 msec^2), acquiring its lowest power in the afternoon-early evening hours (1300h-1900h , 91±23 msec^2) and gradually increasing during the night (1900h-0100h, 214±50 msec^2) (Figure 8.1 right panel). The LF component during the night and early morning (0100h-0700h) was significantly higher (ANOVA, $p<0.008$, Table 8.2) than the periods of the day with the highest physical and mental activity (0700h-1300h and 1300h-1900h).

The LF/HF ratio was at its highest while subjects were asleep (0100h-0700h, 4.7±0.9), decreasing as subjects awoke (lowest value 1300h-1900h, 3.3±0.8, Figure 8.2). A significant inverse correlation ($r=-0.68$, $p<0.02$) was found between the severity of heart failure, estimated by the exercise tolerance during upright bicycle exercise, and sleep/wake LF/HF ratio (Figure 8.3).

TABLE 8.2. Circadian Variation in the Frequency Domain Measures of Heart Rate Variability in Patients with Chronic Heart Failure; Effects of Physical Training

	LF power (msec^2)		**HF power** (msec^2)		**LF/HF** ratio	
Time	Detraining	Training	Detraining	Training	Detraining	Training
7am-1pm	159±43	351±146	89±35	164±75**	4.1±0.7	3.0±0.4
1pm-7pm	91±23	154±42	39±14	133±67*	3.3±0.8	2.9±0.6
7pm-1am	214±50	298±96	111±54	198±100**	4.6±1.1	3.2±0.6
1am-7am	361±83††	535±202	126±47	227±115	4.7±0.9	4.0±0.8

LF = low-frequency; HF = high-frequency.
mean±SE; * $p<0.05$ and ** $p<0.01$ detraining vs training, †† $p<0.01$ 1am-7am vs 7am-1pm and 1pm-7pm.

iii. Effects of Physical Training on Heart Rate Variability Measures in Patients with Chronic Heart Failure

1. *In the time domain* (Table 8.3). Training induced significant increases in SDNN index (39.3±3.7 msec vs 33.4±2.7 msec, $p<0.01$, Figure 8.4), rMSSD (25.9±5.3 vs 19.2±3.2 msec, $p<0.03$) and pNN50% (6.8±3 vs 3.2±1.3, $p<0.05$), indicating an enhancement in vagal tone, with a concomitant reduction in heart rate (Figure 8.4 shows the changes in SDNN index induced by training in each patient).

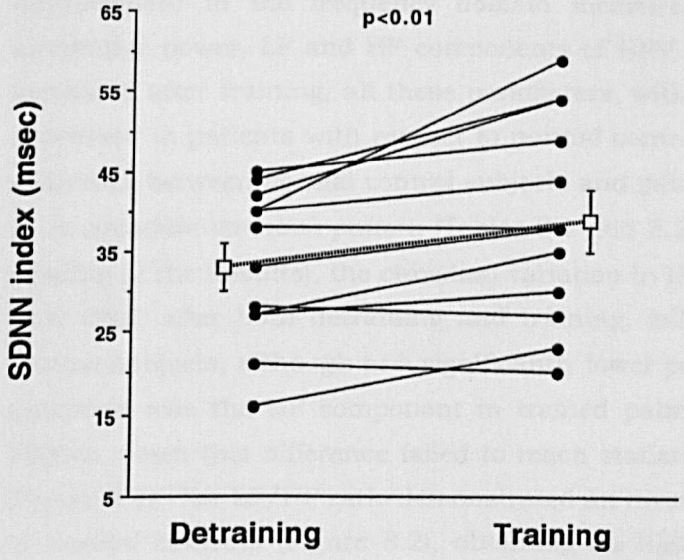

Figure 8.4. Graph of individual (and mean±SEM) mean of all 5-min standard deviations of R-R intervals (SDNN index) in patients with chronic heart failure, after detraining and after training. Note the increase of SDNN index with physical training, indicative of enhancement of vagal activity (** $p<0.01$).

2. *In the frequency domain* (Table 8.3). There were significant increases in LF (from 169±35 $msec^2$ after detraining to 279±88 $msec^2$ after training, $p<0.05$), HF (from 70±26 $msec^2$ to 160±81 $msec^2$, $p<0.01$) and total power (from 535±95 $msec^2$ to 847±225 $msec^2$, $p<0.01$) associated with significant decreases in the LF/HF ratio (from 4±0.7 after detraining to 3.3±0.5 after training, $p<0.05$), confirming the training-induced shift away from sympathetic and towards vagal predominance.

iv. Physical Training Effects on the Circadian Pattern of Heart Rate Variability in Patients with Chronic Heart Failure

Despite the significant overall increase throughout the day in the high-frequency component and decrease in the LF component and LF/HF ratio, training produced no alterations in the circadian pattern of the frequency domain measures of HRV. Also after training the power of the HF component was highest during the first hours of the day (0100h-0700h, 227±115 $msec^2$) progressively decreasing to its lowest value during the afternoon-early evening hours (1300h-1900h, 133±67 $msec^2$) (Table 8.2, Figure 8.1 left panel). Similarly, the power of the LF component reached its highest value during sleep (535±202 $msec^2$) and its lowest during the afternoon-early evening hours (154±42 $msec^2$), with the intermediate power progressively decreasing (0700h-1300h, 351±146 $msec^2$) and increasing (1900h-0100h, 298±96 $msec^2$) respectively (Table 8.2, Figure 8.1 right panel). A similar pattern was also

followed by the LF/HF ratio, which showed the highest value during sleep (4.0±0.8) and the lowest during the period from 1300h-1900h (2.9±0.6) (Figure 8.2).

v. Comparisons Between Patients with Chronic Heart Failure and Normal Controls

1. *In the time domain* (Table 8.3). In patients with CHF, the time domain measures of HRV (SDNN index, rMSSD and pNN50%), although increased by physical training, still remained far below the values of the same parameters in the control group.

2. *In the frequency domain* (Table 8.3). Similarly, training produced a significant improvement in the frequency domain measures of HRV by increasing 24-hour total autonomic power, LF and HF components of HRV and reducing the LF/HF ratio. Although increased after training, all these parameters, with the exception of LF/HF ratio, remained depressed in patients with respect to normal controls. Low/high frequency ratio showed no difference between normal control subjects and patients with CHF after training.

3. *In circadian variation pattern* (Tables 8.1 and 8.2). As described above (under the second heading of the Results), the circadian variation in HF and LF components of HRV in patients with CHF, after both detraining and training, followed a pattern similar to that seen in normal subjects, although at a significantly lower power throughout the 24-hour period. The exception was the HF component in trained patients during the early afternoon (1300h-1900h), when this difference failed to reach statistical significance with respect to controls (Figure 8.1). The LF/HF ratio demonstrated an inverse pattern of variation in CHF compared to normal subjects (Figure 8.2), obtaining its highest values during sleep and its lowest during the afternoon-early evening period of increased activities.

TABLE 8.3. Effects of Physical Training on Time and Frequency Domain Measures of Heart Rate Variability; Comparisons with a Control Group of Normal Subjects

Measures	Detraining	Training	Normal Controls
Heart rate	80.2±3.1	77.3±3.0*	72.3±2.3
SDRR (msec)	97.8±7.2	105.8±8.3	141±11.7†
SDANN (msec)	88.6±7.0	93.6±8.2	126±12.5†
SDNN index (msec)	33.4±2.7	39.3±3.7*	57.5±47†
rMSSD (msec)	19.2±3.2	25.9±5.3*	32.8±3.0
pNN50%	3.2±1.3	6.8±3.0*	11.6±2.3
Total power ($msec^2$)	535±95	847±225**	1878±325
Low-Frequency ($msec^2$)	169±35	279±88*	749±150†
High-Frequency ($msec^2$)	70±26	160±81**	237±47
Low/High-Frequency ratio	4.0±0.7	3.3±0.5*	3.5±0.5

SDRR = standard deviation of all R-R intervals; SDANN = standard deviation of 5-min mean R-R intervals; SDNN index = mean of all 5-min standard deviations of R-R intervals; rMSSD = root mean square of the differences of succesive R-R intervals; pNN50% = percentage difference between adjacent normal R-R intervals >50 msec.
Mean±SE; * $p<0.05$ and ** $p<0.01$ between detraining and training, † $p<0.05$ and †† $p<0.01$ between training and normal control subjects.

E. Discussion

Time and frequency domain measures of HRV, derived from short ECG recordings during supine-rest in the laboratory environment, improve after physical training in patients with CHF (Coats *et al.*, 1992a). There may be a 'beneficial feedback' between improvement in cardiovascular function and shifts in autonomic balance from sympathetic to vagal predominance, given the significant correlations between enhanced exercise tolerance after training and changes in various time domain measures of autonomic control (Adamopoulos *et al.*, 1992).

We now confirm that these findings hold for analysis of HRV over the full 24-hour period. We are also now in a position to suggest that physical training by increasing time (SDNN index, rMSSD, pNN50%) and frequency (HF power) domain descriptors of vagal tone acts through shifting autonomic balance towards vagal predominance. Furthermore, we provide detailed information regarding the effects of training on all measures of time and frequency domain of HRV, some of which (SDNN index, LF, HF, LF/HF ratio) have been shown to be strongly and independently associated with early or late death during follow-up after myocardial infarction (Farrel *et al.*, 1991; Bigger *et al.*, 1993). Thus physical training, by reversing, at least partially, all the parameters describing HRV in the time and frequency domain, may acquire prognostic significance in patients with moderate to severe CHF. These findings may reflect beneficial changes in central autonomic control and/or in baroreflex-cardiopulmonary receptor sensitivity, in keeping with the increased vagal tone found after endurance exercise training in sedentary healthy or hypertensive subjects (Somers *et al.*, 1986a; Pagani *et al.*, 1988). However, the values of these parameters, even after training in CHF remain below the corresponding values in a control group of normal subjects, indicating that exercise training only slightly improves impaired autonomic balance in patients with CHF.

Our study also throws light on the circadian variation of these parameters in both normal controls and patients with CHF before and after a non-pharmacological intervention. The circadian variations of the LF and HF components of HRV throughout the day are similar in normal subjects and in patients in both training and detraining conditions, indicating that the training-induced improvement in time and frequency domain measures of HRV is an inherent phenomenon rather than due to increased physical activity after training.

The preservation of a circadian variation of LF and HF components between patients after both training and detraining also suggests that the syndrome of heart failure is characterised by a resetting of autonomic balance rather than autonomic neuropathy. In diabetic patients, particularly if autonomic neuropathy is present, there is a loss of day-night modulation of the autonomic tone (Bernardi *et al.*, 1992), as opposed to an impaired sympatho-vagal balance but with preserved circadian oscillations seen in patients with CHF.

We observed that increased HF and LF components were present during the night both in normal subjects and in patients, either after detraining or after training. High-frequency

and some low-frequency oscillations, that occur at a higher level during sleep, can be explained by an enhanced vagal tone [as vagal input has been demonstrated to be a major contributor to HF power and has also been involved in the origins of LF (Pomeranz *et al.*, 1985; Pagani *et al.*, 1986; Malliani *et al.*, 1991)] during the night.

Instead, the LF/HF ratio showed an inverse pattern of distribution in patients with CHF after detraining and even after training, with respect to normal controls, obtaining higher values during the night (Figure 8.2). This discrepancy may be explained by the different factors that contributed to the circadian variations in the two groups of subjects. Throughout the day in normal subjects, compared to CHF patients, there is a higher autonomic oscillatory power, involving both LF and HF components (as clearly shown in Figure 8.1). During the day the vagal tone is partially reduced, due to more intensive mental and physical activity, with consequent predominance of low frequency oscillation (mainly sympathetically mediated) over HF components (mainly vagally mediated). During sleep the vagal tone is unmasked and therefore markedly enhanced, contributing substantially to the higher frequency power during the night and the lower LF/HF ratio (Figure 8.3). In patients with CHF, a predominance of LF over HF components during the night induced an inversion of the circadian rhythmicity of sympatho-vagal balance. This suggests increased sympathetic activity overnight, as a result of increased activity in afferent sympathetic fibres with sensory endings in the pulmonary veins, perhaps excited by nocturnal pulmonary congestion (Malliani and Pagani, 1983). An alternative explanation for the relatively elevated LF component during the night in patients with CHF could be the absence these oscillations during the day, due to extreme adrenergic hyperactivity (Eckberg *et al.*, 1971; Leimbach *et al.*, 1986; Ferguson *et al.*, 1992), which is translated into reduced LF at that time. This explanation is reinforced by the finding that the more severe the heart failure, as estimated by exercise tolerance, the lower the LF and LF/HF ratio values (Figure 8.3). When the sympathetic system is highly activated, its oscillations in regulating heart rate are either absent or appear extremely low in the spectra of HRV and cannot be detected as a LF component (Malliani *et al.*, 1991). In CHF or in some patients after myocardial infarction the sinus node is likely to be unresponsive to neural modulation. The crucial point is that, while normally no coherence exists between respiration and low-frequency, the very slow oscillations occuring during CHF (visible also in analog recordings) are in synchrony with respiratory breathing (Saul *et al.*, 1988).

The clinical manifestations and cardiovascular complications of coronary artery disease, such as transient myocardial ischaemia (Rocco *et al.*, 1987), non-fatal myocardial infarction (Muller *et al.*, 1985), ventricular arrhythmias (Raeder *et al.*, 1988) and cardiac death (Muller *et al.*, 1987), are known to have a peak occurence between 0600 am and noon. During this period, as opposed to a "smooth" increase of the HRV parameters during the night, we observed a relatively sharp reduction in the power of LF and HF components of HRV, indicating a shift towards more sympathetic and less vagal predominance during this period. Although we cannot determine whether the changes in autonomic tone are causal or contributory to sudden death in CHF, or simply occur in parallel (Malik and Camm, 1993),

by modifying this pattern of early morning variation in HRV measures we might have an effect in the incidence of arrhythmias. It is known that stimulation of the vagus has a powerful antiarrhythmic effect in both animal models (Vanoli *et al.*, 1991) and in humans with ventricular tachycardia (Waxman and Wald, 1977). A reduction in vagal tone, expressed by the HF component of HRV, for a few minutes before the onset of non-sustained ventricular tachycardia has been reported (Ponikowski *et al.*, 1993). Physical training in CHF, by enhancing vagal tone, induced not only increases in R-R interval and HRV but also prolongation of Q-T duration, as previously reported in normal trained subjects (Palatini *et al.*, 1987). This change in the recovery of ventricular excitability may have beneficial effects in the suppression of ventricular arrhythmias. It has also been recently shown that low doses of scopolamine can cause a sustained increase in cardiac vagal tone and improve autonomic indices associated with a high mortality in patients in the acute phase of myocardial infarction (Casadei *et al.*, 1993) or with CHF (La Rovere *et al.*, 1994).

i. Clinical Implications

Measurements of HRV are powerful, non-invasive, prognostic markers and have been used as a supplement to other markers in discriminating between low and high risk groups of patients with CHF (Binder et al., 1992). In this study, exercise training in CHF improved measures of HRV in relation to both time and frequency whilst preserving its circadian variation. Given the prognostic significance of reduced HRV and vagal activity, these beneficial changes may lessen the predisposition to ventricular arrhythmias and reduce mortality in CHF.

A description of the circadian pattern of HRV in CHF, a syndrome with abnormal neurohormonal regulation, might be helpful in planning therapeutic strategies and/or modifying existing ones for this category of patients. The sudden rise of sympathetic activity and particularly the reduction of vagal tone in the morning hours can be modified through various therapeutic interventions, thus preventing the enhanced rate of cardiovascular complications during this period.

Chapter IX

Pulsing ß-Stimulant Therapy Up-Regulates ß-Adrenoceptors and Enhances Chronotropic Responsiveness in Chronic Heart Failure

A. Abstract

Objectives and Background. In animal, intermittent sympatho-mimetic stimulation with dobutamine produces benefits analogous to those of physical conditioning. Longer intermittent and continuous ß-stimulant or other inotropic therapies have, however, not been successful in managing patients with CHF. The purpose of this study was to reconsider the role of ß-receptor stimulants in patients with severe CHF by changing the method of administration to intermittent, very short duration pulsed inotrope therapy (PIT).

Methods. To investigate whether intermittent, very short-duration PIT may be able to produce long-lasting pharmacological conditioning in CHF, we studied 10 patients (mean age 64±2 years) with stable moderate to severe CHF (ejection fraction: 23±3%), and 10 control patients matched for age and severity. We infused dobutamine sufficient to raise heart rate to 70-80% maximum and maintained the infusion for 30 min per day, 4 days per week for 3 weeks with major end-points lymphocyte ß-receptor density, autonomic control and exercise tolerance. The same parameters were re-evaluated immediately and 6 weeks after the completion of PIT and in the control group of patients.

Results. Pulsed inotrope therapy increased exercise tolerance (from 10.4±1.2 at baseline to 13±1.5 min at 3 weeks, $p<0.001$, 95% CI for difference 1.6 to 3.9) and reduced peripheral vascular resistance (from 19.8±3 to 17.7±2 mmHg.min.L^{-1}, $p<0.05$, -4.1 to -0.1). Pulsed inotrope therapy produced significant increases in lymphocyte ß-receptor density (from 502±110 to 1200± 219 ß-receptors/cell, $p<0.02$, 258 to 1138) and chronotropic responsiveness to exercise (change in heart rate rest to peak exercise +51±3 to +57.5±4 beats per min, $p<0.01$, 2.9-10.1) or to dobutamine (change in heart rate rest to peak infusion +40.6±4 to +44.7±4 beats per min, $p<0.05$). These findings were associated with reduced sympathetic activity, as estimated by the reduction of plasma NA concentrations (405±47 to 279±32 pg/ml, or 2.39±0.28 to 1.65±0.19 nmol/L, $p<0.05$) and the reduction of the low/high-frequency (LF/HF) ratio ($p<0.002$), an index of sympathetic tone derived by power spectral analysis of HRV. The patients' symptoms were also improved. By contrast, no change in autonomic function or exercise capacity was seen in the control group. Six weeks after the completion of PIT, sympathetic tone remained lower compared to pre-PIT, and exercise capacity was higher (11.3±1.3 min, $p<0.02$).

Conclusions. Short duration PIT induces pharmacological conditioning with improved symptoms, autonomic balance, exercise tolerance, ß-receptor up-regulation and enhanced chronotropic responsiveness in patients with CHF. These beneficial effects persisted for at least 6 weeks after PIT.

B. Introduction

Chronic heart failure is a complex syndrome with abnormalities in many systems. In addition to cardiac muscle disease, other (non-cardiac) abnormalities may contribute to the associated symptoms, exercise intolerance and high mortality rate. Specific strategies to reverse these abnormalities have not yet been discovered. One important abnormality, which contributes to exercise intolerance in CHF, is ß-receptor down-regulation (Bristow *et al.*, 1982) and, as a consequence, a reduced ability to mount an adequate heart rate response to exercise (chronotropic incompetence) (Colucci *et al.*, 1989).

Long-term inhibition of angiotensin-converting enzyme (ACE) (Maisel *et al.*, 1989; Townend *et al.*, 1993) and ß-blocker therapy (Michel *et al.*, 1988) have been reported to up-regulate ß-receptors. Although sympathetic withdrawal cannot be excluded, this up-regulation may be secondary to a general improvement in condition with ACE inhibition, and may only be possible in a minority of the affected patients in the case of ß-blockade. No specific treatment to up-regulate ß-receptors is available.

The mechanism of ß-receptor down-regulation is thought to be persistent ß-stimulation by the sympathetic hyperactivation known to occur in CHF. It is not known whether pulsatile rather than continuous chronic ß-receptor stimulation would cause less down-regulation but evidence from receptor-agonist systems suggests the possibility.

Experiments in animals, in the early 1980's, have shown that intermittent sympathomimetic stimulation with dobutamine can produce beneficial changes analogous to the effects of physical training (Leier *et al.*, 1982). As did physical training (Coats *et al.*, 1992a), dobutamine infusions improved exercise performance and reduced resting heart rate, exercise-provoked increases in heart rate, arterial blood lactate, plasma renin activity and plasma NA concentration (Liang *et al.*, 1979). These encouraging results led to studies in patients with severe CHF; the treatment produced significant improvements in resting haemodynamics, exercise tolerance and symptoms which persisted for weeks (Liang *et al.*, 1984). Long-term ß-stimulant or other inotrope therapy has not, however, been successful in management of CHF for two main reasons: a) a tolerance to the inotropic stimulation, which in the case of ß-stimulants is substantially related to ß-receptor down-regulation (Tohmeh and Cryer, 1980; Bobik *et al.*, 1981) and b) an increased frequency of ventricular arrhythmias, which may increase mortality (Dies *et al.*, 1986; Packer *et al.*, 1991).

There was an important difference, however, between the animal and the human studies, which has been ignored. The short infusions (1-2 hours) in animals were replaced by much longer infusions (>4 hours to 3 days) in patients, and the duration may be

important. Evidence from exercise studies shows that interval training (shorter bursts of activity interspersed with periods of lower activity) is more effective in producing fitness effects than single bouts of prolonged exercise (Samek *et al.*, 1990). Also ß-receptor activity is reduced when exercise is maintained for more than 1 hour continuously (Mäki 1989), whereas it was not changed or even increased with shorter bursts of exercise (Butler *et al.*, 1983; Mäki *et al.*, 1988). Similarly, continuous pharmacological ß-receptor stimulation with infusions lasting longer than 4 hours results in ß-receptor down-regulation but this does not occur after shorter infusions (Tohmeh and Cryer 1980). Thus, the duration of ß-receptor stimulation may be important in determining whether receptor down- or up-regulation is produced.

We have attempted to imitate pharmacologically the stimulus to the $ß_1$-receptors of the cardiac myocyte produced by physical training. Short periods (20-30 min) of high-level exercise are replaced with short bursts (30 min) of pharmacological ß-adrenergic stimulation with dobutamine. Thus, we aimed to determine whether short duration pulsed inotropic therapy induces a pharmacological conditioning effect without i) ß-receptor down-regulation and ii) the development of pharmacological tolerance.

C. Methods

i. Study Population

Twenty patients (19 male, 1 female) with stable CHF (17 secondary to ischaemic heart disease and 3 with dilated cardiomyopathy) gave informed consent for this trial, which was approved by the local ethics committee. The ischaemic etiology was documented by previous myocardial infarction and/or coronary arteriography. No patient had symptomatic angina or electrocardiographic evidence of ischaemia limiting exercise at the time of the study. No patient had evidence on Holter monitoring of complex ventricular arrhythmias. Patients were limited by symptoms of dyspnoea or fatigue only and were able to reach a respiratory exchange ratio of at least 1. Pharmacological treatment was stable for 3 months before and during the study in all subjects.

Clinical data for the patients who received pulsed inotrope therapy (PIT group) and the 10 control patients are given in Table 9.1. The control patients were matched for age and severity individually to PIT group patients by means of a stratified randomisation schedule. Patients in the PIT group were aged 64±2 years, their radionuclide left ventricular ejection fraction was 23±3% and peak oxygen uptake 12.4±0.8 ml/kg/min and in the control group were aged 63±1 years, their radionuclide left ventricular ejection fraction was 19±3% and peak oxygen uptake 13.8±0.6 ml/kg/min. There were no significant differences in clinical status (New York Heart Association class or exercise tolerance) or current treatment [all subjects were taking diuretics (median frusemide dose 80 mg), all were on ACE inhibitors and only 1 (PIT group) on digoxin] between the patient groups at baseline.

TABLE 9.1. Individual Patient Demographic Data

Patient	**Age** (years)	**Drugs**	**EF** (%)	**VO_2max** (ml/kg/min)	**Ex-Dur** (min)	**NYHA** class
PIT group						
1	65	D; I	16	9.2	8	III
2	62	D; I	39	13.9	14	III
3	67	D; I	34	17	19	II
4	69	D; I; Dig	12	8.6	7	IV
5	66	D; I; A	15	12.6	9.5	III
6	63	D; I	17	10.8	9.3	III
7	67	D; I	NA	10.5	7	III
8	68	D; I	22	13.6	10.5	III
9	50	D; I	28	13.5	7.5	III
10	66	D; I	26	14.2	12	III
Mean (SE)	64±2		23±3	12.4±0.8	10.4±1.2	
Control group						
1	67	D; I	12	15.4	14.7	III
2	64	D; I	35	15.9	16.5	II
3	66	D; I	22	15.7	14.5	III
4	69	D; I;	NA	13.5	10.5	III
5	59	D; I;	13	14.0	16	III
6	67	D; I	28	13.8	12.1	III
7	60	D; I	19	13.2	13	III
8	54	D; I	14	11.1	6.3	III
9	64	D; I; A	9	13.7	12.4	III
10	64	D; I	15	11.6	9	III
Mean (SE)	63±1		19±3	13.8±0.6	12.5±1.1	

D = diuretics; I = angiotensin converting enzyme inhibitors; A = antiarrhythmics; Dig = digoxin; EF = ejection fraction; VO_2max = peak oxygen uptake; Ex-Dur = upright bicycle exercise duration; NYHA = New York Heart Association functional class, NA = not available

On entry to the trial, all patients underwent 2-4 weeks of familiarisation and baseline evaluation during which reproducible exercise and autonomic tests were obtained (three consecutive recordings with no more than 10% difference in exercise tolerance or HRV measures in the frequency domain).

Subsequently, PIT patients were studied before and after 3 weeks of dobutamine infusions and 6 weeks after the end of the intervention to look at the long-term duration of the dobutamine-induced effects on exercise tolerance and autonomic function. The control patients were studied before and after 6 weeks of normal physical activities only. All tests were carried out by staff unaware of treatment allocation.

ii. Pulsed Inotrope Therapy (PIT)

The dobutamine dose was titrated to keep the patients' continuously monitored heart rate in the range of 70-80% of their previously determined maximal heart rate (as measured by upright bicycle exercise during the familiarisation phase). This was achieved by giving incremental infusions (5 μg/kg/min increments every 5 min) through a pump syringe until the desired heart rate had been reached (15 μg/kg/min in 9 patients and 10 μg/kg/min in 1); this dose was maintained for a total infusion period of 30 min. Pulsed inotrope therapy patients attended 4 days a week for 3 weeks for a total of 12 sessions of PIT (intravenous dobutamine). On the first 2 and the last 2 infusion days we also increase the dobutamine infusion rate to a "maximum" dose: the rate of dobutamine infusion required to reach the previously determined maximal exercise heart rate (25 μg/kg/min in 6 patients and 20 μg/kg/min in 4). We compared the haemodynamic response to acute dobutamine infusion between the first 2 and the last 2 infusions to assess the effects of PIT on the response to dobutamine. A single 30-min dobutamine infusion was also given 6 weeks after the completion of the infusion "training" period to look at the longer term effects of the PIT on exercise tolerance and autonomic balance variables.

iii. Lymphocyte ß-adrenoceptor Density

Lymphocyte ß-adrenoceptor density was assayed at least 48 hours after the last period of dobutamine infusion or exercise test, after 30 min supine rest. On each occasion, lymphocytes were isolated from 40 ml of whole blood according to the method of Boyum (1968). The lymphocytes were then divided into 16 aliquots (approximately 1-2 x 10^6 cells each), and suspended in incubation medium (RPMI 1640 containing 20% calf serum Gibco Biocult Ltd, UK). The cells were then incubated with 7 concentrations of (^{125}I) ICYP (Amersham International, UK) between 5 and 50 pmol/l, with and without propranolol (Sigma chemicals Ltd, UK) at a concentration of 0.3μM. In all cases, 16 incubation tubes were prepared so that on each occasion 2 could be used to check viability of cells before and after incubation. The 16 incubation bottles were then placed on a gyratory mixer and left in the cold room (4° C) for 20 hours. When the incubation was completed, the contents of each bottle were poured over Whatman GF/C filters (Whatman LabsalesLtd, UK), each bottle was then washed twice with 12.5 ml ice-cold buffer. After drying, the radioactivity on the filters was counted using an Innotron Hydragamma16 Scintilation Spectrometer at counting efficiency of 80%. Specific binding was then calculated by subtracting non-specific binding (tubes containing propranolol) from the corresponding total binding (tubes without propranolol). The ß-adrenoceptor density was then calculated by Scatchard analysis.

iv. Exercise Testing

Exercise tests were performed on a Tunturi Professional Ergometer (Tunturi, Finland). All tests were performed in the fasted state before daily medication had been taken and all were conducted by a neutral "blinded" observer. Bicycle exercise tests were performed in the

upright position in 5-min stages, with 25-W increments to the limit of tolerance, with 1-min average measurements of respiratory gas exchange. Oxygen consumption and CO_2 production were measured during the test using standard formulas (Coats *et al.*, 1990). On the same day, after 1 hour rest, a second exercise test was done with the subject supine during which haemodynamic measurements were made at rest and at the end of each 5-min/25-W incremental stage of the supine bicycle exercise test (Elema-Schonander, AM 368, Stockholm, Sweden). Pulsed wave Doppler ultrasonic measures of ascending aortic blood velocity from the suprasternal approach were made using a Pedof Doppler ultrasound generator (Vingmed, Norway) and our own laboratory made computer-based Fast Fourier Transform spectral analyser (Murphy *et al.*, 1988). Using the intensity-weighted mean frequency in each 5-msec time bin, stroke distance (the integral of velocity and time for the ejection period) was calculated. Using standard formulae and an echocardiographic measurement of aortic cross-sectional area (leading edge to leading edge, immediately distal to the sinus of Valsalva), stroke volume was calculated; this method of measuring volume flow has been validated in our laboratory and proved to be accurate (standard deviation of differences from electromagnetic catheter estimate = 4%) (Coats *et al.*, 1992b). Blood pressure was measured by a previously validated automatic sphygmomanometer (Copal UA 241, Takeda Medical, Tokyo) (Coats *et al.*, 1989). Total peripheral vascular resistance was calculated as mean blood pressure divided by cardiac output.

v. Twenty-Four-Hour Electrocardiographic Monitoring

Twenty four-hour electrocardiographic monitoring was performed using a 2-channel recorder (Oxford Medilog II, Oxford Med. Instruments). Modified V_1 and V_5 leads were recorded. Two channel qualitative and quantitative electrocardiogram analysis was performed using a computerised non-triggered template system consisted of a Z80A preproccesor and DEC-LSI master, developed and validated in our laboratory (Rossi *et al.*, 1983). Analysis of the recordings was performed blind to other patient characteristics.

vi. Autonomic Function Assessment

1. *Power Spectral Analysis of the R-R Variability*

Signal acquisition and power spectral analysis. After 30-min bed rest in a quiet darkened room 640 consecutive heart beats (Lead V_5) were recorded on a Store 4 Racal-Thermionic FM tape recorder (Southampton, UK), at 15/16 inches/sec. The tape-recorded data were digitised off-line by a 12-bit analog-to-digital converter (NB-MIO-16 board, National Instruments, Austin, Tx) at a sampling rate of 500 samples/sec. The converter was connected to a MacIntosh IIcx computer (Apple Inc., Coupertino, Ca) equipped with 5Mb RAM memory and a 60Mb hard disk. A "C" language programme identified R wave peaks in each sequence. Power spectral analysis of the R-R interval signal was performed by an autoregressive model on 256 consecutive heart beats (Kay and Marple, 1981). Model coefficients were evaluated according to a modification of the Burg algorithm and model

order was assessed by Akaike criteria (Pagani *et al.*, 1986); a model order between 9 and 13 was found to be adequate in most cases. Spectral components were obtained by a decomposition method previously described (Kay and Marple, 1981; Pomeranz *et al.*, 1985), which was also used to measure the area below each spectral peak. The power spectrum shows two separate peaks: the higher frequency (HF) peak is related to respiration; the low-frequency (LF) peak appears unrelated to any respiratory event and has been found to reflect changes in sympathetic tone (Pomeranz *et al.*, 1985; Pagani *et al.*, 1986). We evaluated the power of the harmonic components in the range 0.03 and 0.14 Hz (LF component) and those in the range 0.18 and 0.40 Hz (HF component). To simplify comparison of spectra, the percentage of each spectral component as a proportion of the total oscillatory power was calculated [normalised units (nu)], together with the logarithmic absolute values for both LF and HF spectral components. The amount (relative or absolute) of the HF component has been considered as a clinical index of parasympathetic activity, whereas the amount (relative or absolute) of the LF component and the LF/HF ratio have been considered as indices of sympathetic activity (Pagani *et al.*, 1986).

2. *Noradrenaline Kinetics*

Noradrenaline kinetics were measured according to the techniques of Esler *et al.* (1979). 1-[2,5,6-^{3}H]NA (New England Nuclear, Boston Massachusetts) was given intravenously as a bolus [12 microCi (0.44 MBq)] followed by constant infusion [0.7 microCi (0.026 MBq)/min^{-1} m^{-2}] for up to 60 min. Arterial and venous blood samples were collected at rest and at submaximal infusions of dobutamine (15μg/kg/min) in the control phase, after the PIT period and 6 weeks following the completion of PIT. The blood samples were analysed for plasma NA using high performance liquid chromatography and NA plasma clearance was then measured as has been previously described (McCance and Forfar, 1989a). The normal range of plasma NA in our laboratory is 0.71-1.77 nmol/L.

vii. Symptom Assessment

A modified Likert symptom questionnaire was used to assess severity of symptoms relevant to CHF at control phase and after 3 weeks of dobutamine infusions. Patients were asked to grade the severity of breathlessness, fatigue and chest pain on a scale of 1-7. For all questions an improvement in symptoms is indicated by a lower score.

viii. Statistical Analysis

Analysis of variance was used for comparisons in heart rate, upright bicycle exercise time, stroke volume, the LF/HF ratio in both logarithmic (ln) absolute values and normalised units (nu) and in plasma NA concentrations (corrected for multiple comparisons by Scheffe's procedure). The non-parametric Wilcoxon signed-rank test was used for comparisons in lymphocyte ß-adrenoceptor density, peak oxygen uptake, peripheral vascular resistance and in LF and HF components of power spectral analysis of HRV in both logarithmic absolute

values and normalised units between control phase, PIT period and 6 weeks following PIT. Results are expressed as means±SEM.

D. Results

i. Control Group Measurements

Autonomic balance and exercise tolerance were assessed in 10 age-matched control patients with severe CHF on 2 occasions with 6 weeks separating each other. In the control group

TABLE 9.2. Results in Control Group at Baseline and 6 Weeks

	Baseline	**6 weeks**	**95% CI for difference**
Exercise tolerance (min)	12.5±1.1	12.8±1.2	-0.3 to 0.9
Resting heart rate (beats per min)	79.2±3.7	80.2±3.8	-4.2 to 6.2
Plasma noradrenaline (nmol/L)	2.18±0.32	2.03±0.34	-0.57 to 0.27
Low/High-Frequency ratio	6.5±1.1	6.7±1.5	-6.7 to 1.1

Values are expressed as mean±SEM

there were no significant changes from baseline to 6 weeks in exercise tolerance, resting heart rate, plasma NA and LF/HF ratio (Table 9.2), indicating no period effects in these patients in autonomic control and exercise capacity.

ii. Pulsed Inotrope Therapy Group Measurements

1. *Exercise Tolerance and Ventilatory Function*

In the PIT group, upright bicycle exercise time increased significantly by 25% and average peak oxygen uptake by 10.1% (Table 9.3) after 3 weeks of PIT. Individual patient data on the changes in exercise time are demonstrated in Figure 9.1. Pulsed inotrope therapy produced a significant reduction in submaximal heart rate at 25-W and 50-W during upright bicycle exercise but no change in peak exercise heart rate (Table 9.3). The intervention produced a significant increase in heart rate (chronotropic) reserve; the difference between basal (after 30 min supine rest) heart rate and peak upright bicycle exercise heart rate rose significantly by 12.7%.

2. *ß-receptor Density*

There was a significant increase in lymphocyte ß-adrenoceptor density with PIT in all 10 patients (Figure 9.2). This change indicates not only avoidance of down-regulation but also up-regulation of ß-receptor number.

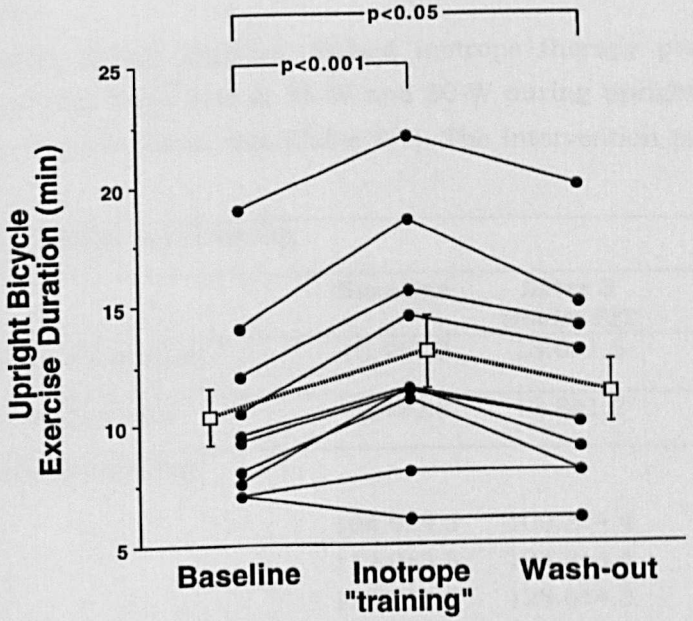

Figure 9.1. Graph of individual and mean±SEM patient upright bicycle exercise durations at baseline, after 3 weeks of pulsed inotrope therapy (inotrope "training") and 6 weeks later (wash-out); $p<0.001$ for comparisons between baseline and after the inotrope "training" period, $p<0.05$ for comparisons between baseline and wash-out period.

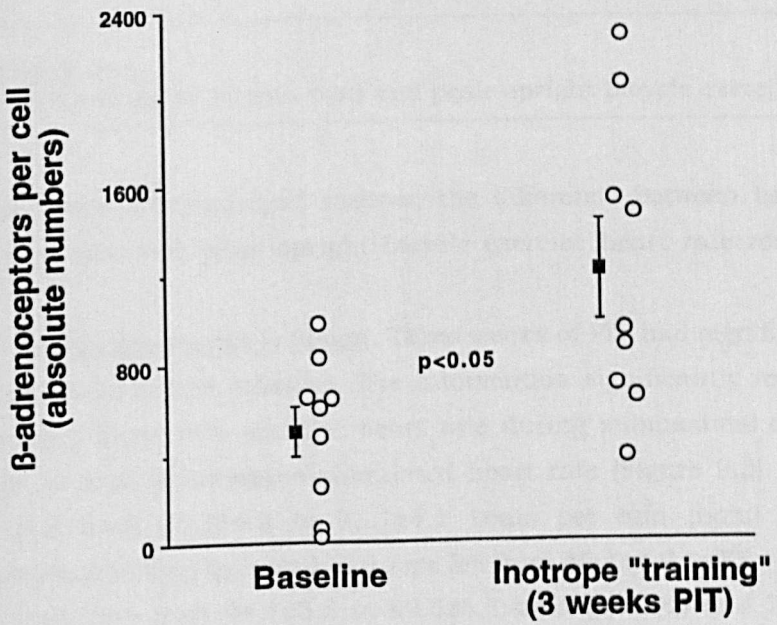

Figure 9.2. Graph of the effects of short duration pulsed inotrope therapy on lymphocyte ß-adrenoceptor density at baseline and after 3 weeks of pulsed inotrope therapy (inotrope "training"). The ß-adrenoceptor density has been expressed in absolute numbers of ß-adrenoceptors per cell.

3. *Autonomic Function*

Heart rate during bicycle exercise. Pulsed inotrope therapy produced a significant reduction in submaximal heart rate at 25-W and 50-W during upright bicycle exercise but no change in peak exercise heart rate (Table 9.3). The intervention produced a significant

TABLE 9.3. Cardiac Variables in PIT Group

	Baseline	**After 3 weeks PIT**	**p-value**	**95% CI for difference**
Upright bicycle exercise time (min)	10.4±1.2	13.0±1.5	<0.001*	1.6 to 3.6
Peak oxygen uptake (ml/kg/min)	12.4±0.8	13.8±1.2	<0.05†	
Heart rate during exercise testing (beats per min)				
Submaximal 25-W	104.9±4.3	100.3±4.4	<0.002*	-6.6 to -2.6
Submaximal 50-W	112.0±3.5	104.8±4.1	<0.001*	-12.0 to -3.4
Peak	127.1±3.5	128.6±4.3	0.96	-2.5 to 5.5
Heart rate reserve ¶	51.0±3.2	57.5±3.9	<0.01*	2.9 to 10.1
Heart rate difference between basal conditions and peak dobutamine dose (beats per min)	41.0±4.0	45.0±4.0	<0.05*	0.6 to 7.6
Heart rate variability				
Low-Frequency component (normalised units)	66.4±3.3	45.7±4.8	<0.001†	-30.3 to -11.1
High-Frequency component (normalised units)	26.6±3.4	45.7±6.6	<0.003†	6.4 to 31.8
Low/High-Frequency ratio				
normalised units	2.8±0.4	1.3±0.3	<0.001*	-1.0 to -2.0
log absolute values	1.3±0.08	1.2±0.08	<0.002*	-0.16 to -0.40

* ANOVA
† Wilcoxon signed-rank test
¶ Difference between basal (after 30 min rest) and peak upright bicycle exercise heart rate

increase in heart rate (chronotropic) reserve; the difference between basal (after 30 min supine rest) heart rate and peak upright bicycle exercise heart rate rose significantly by 12.7%.

Heart rate during dobutamine infusion. Three weeks of PIT had significant effects on the heart rate during dobutamine infusion. The intervention significantly reduced basal (after 30 min supine rest) heart rate and the heart rate during submaximal dobutamine doses, with no change in peak dobutamine-stimulated heart rate (Figure 9.3). Mean basal heart rate was reduced from 76.1±4.3 to 71.1±4.1 beats per min (bpm) ($p<0.002$). During infusions of dobutamine 5μg/kg/min heart rate fell from 80.1±4.9 to 75.4±4.3 bpm ($p<0.03$), 10μg/kg/min heart rate from 94.7±5.5 to 89.5±5.1 bpm ($p<0.02$) and 15μg/kg/min heart rate from 107.3±6.1 to 103±5.8 bpm ($p<0.03$) after PIT. Peak dose (23.5μg/kg/min) heart rate remained unchanged from 116.7±5.1 to 115.8± 5.7 bpm (p=NS).

Pulsed inotrope therapy also produced a significant increase in the difference between basal and peak dobutamine dose heart rate (Table 9.3), indicating a greater chronotropic reserve to dobutamine stimulation after PIT similar to that seen for exercise (Figure 9.3).

In 24-hour Holter monitoring, no serious or complex ventricular arrhythmias were detected on any of the recordings. There was also no significant change in the rate of early premature beats.

Heart rate variability in the frequency domain. There was a reduction in the LF component associated with an increase in the HF component of HRV and a significant reduction in the ratio LF/HF in normalised units and in logarithmic absolute values (Table 9.3, Figure 9.4), indicating a shift away from sympathetic predominance.

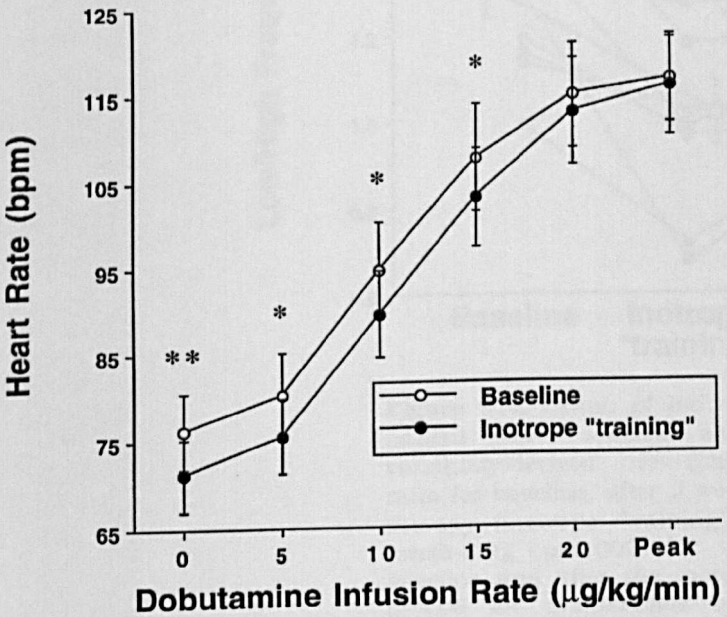

Figure 9.3. Graph of the heart rate response to incremental dobutamine infusions at baseline (open circles) and after 3 weeks of pulsed inotrope therapy (inotrope "training") (closed circles). Note the lower heart rate at baseline (no infusion) after the inotrope "training" period and the same peak heart rate achieved in both baseline and after the inotrope "training" periods; ** p<0.01, * p<0.05.

Noradrenaline kinetics. Pulsed inotrope therapy produced a significant reduction in resting plasma NA concentration (2.39±0.28 to 1.65±0.19 nmol/L, p<0.05 ANOVA; 95% CI for difference -0.21 to -1.28; Figure 9.5) and in peak dobutamine dose plasma NA concentration from 2.49±0.16 to 1.78±0.18 nmol/L (p<0.03). However, there was no change in resting NA clearance (1.1±0.1 to 1.4±0.2 ml/min/m^2, p=NS; 95% CI for difference -0.3 to 0.9).

4. *Peripheral Vascular Resistance.*

Pulsed inotrope therapy produced significant systemic vasodilatation at rest (from 19.8±3.1 to 17.7±2.4 mmHg.min/L, p<0.05 Wilcoxon signed- rank test; -4.1 to -0.1) and at submaximal (15 µg/kg/min) dobutamine infusion (from 10.3±1.3 to 9.2±1.1 mmHg.min/L, p<0.05; -1.9 to -0.3) but no significant change at peak (23.5 µg/kg/min) dobutamine dose (from 9.3±1.3 to 8.6±1.2 mmHg.min/L, p=0.07; -1.5 to -0.1). The haemodynamic responses are shown in Figure 9.6. There was an increase in cardiac output at submaximal dobutamine infusion (from 8.9±1.3 to 10.1±1.1 L/min, p<0.05; 0.3 to 2.1) with no significant change in mean blood pressure. The increased cardiac output was mainly due to increased stroke volume at submaximal dobutamine infusion.

5. *Symptom Assessment.*

There were significant improvements (lower scores better) with PIT in patient-scored symptoms of breathlessness (from 3±0.5 to 1.8±0.3, p<0.05; -2.2 to -0.2) and tiredness (from 3.4±0.5 to 2.6±0.5, p<0.05; -1.5 to -0.1) with no change in the perception of chest pain.

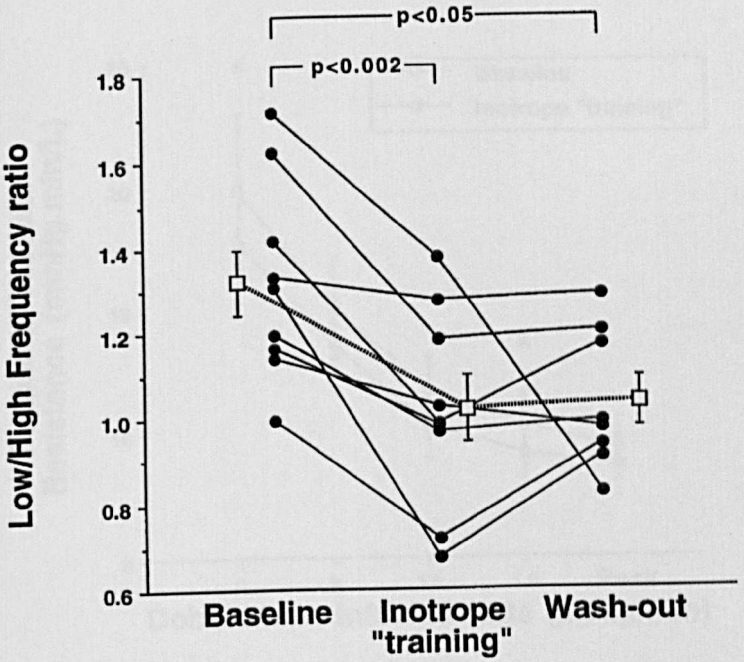

Figure 9.4. Graph of individual and mean±SEM patient power spectral analysis of heart rate variability-derived low-frequency/high-frequency ratio for baseline, after 3 weeks of pulsed inotrope therapy (inotrope "training") and 6 weeks later (wash-out); $p<0.002$ for comparisons between baseline and after the inotrope "training" period, $p<0.05$ for comparisons between baseline and wash-out period.

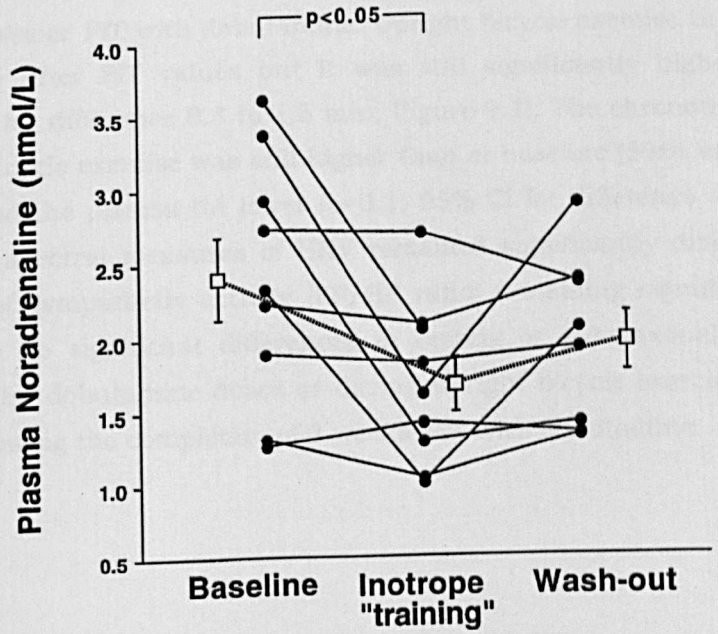

Figure 9.5. Graph of individual and mean±SEM patient plasma noradrenaline concentrations at baseline, after 3 weeks of pulsed inotrope therapy (inotrope "training") and 6 weeks later (wash-out); $p<0.05$ for comparisons between baseline and after the inotrope "training" period.

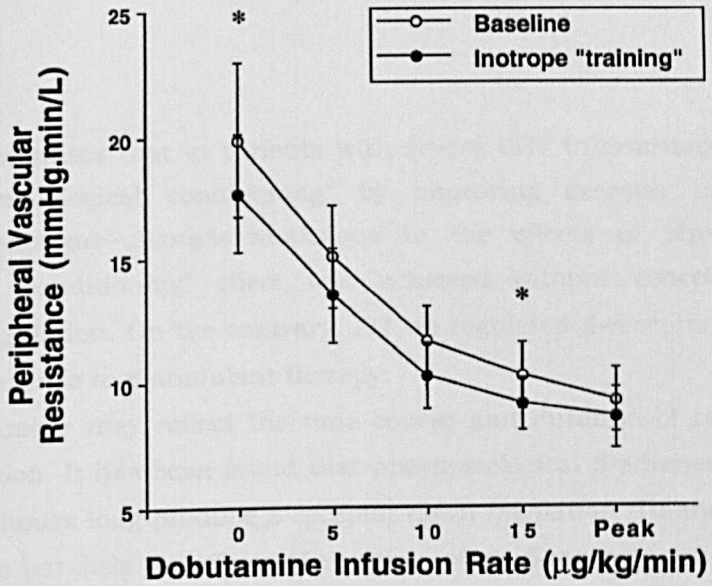

Figure 9.6. Graph of the peripheral vascular resistance response to incremental dobutamine infusions at baseline (open circles) and after 3 weeks of pulsed inotrope therapy (inotrope "training") (closed circles). Note the significant systemic vasodilatation at baseline (no infusion) and at submaximal (15µg/kg/min) infusion after the inotrope "training" period; * $p<0.05$.

iii. Longer-term Effects of Pulsed Inotrope Therapy

Exercise tolerance and autonomic balance parameters were also assessed 6 weeks after the completion of 3 weeks' PIT with dobutamine. Upright bicycle exercise time was shorter than that immediately after PIT values but it was still significantly higher than at baseline ($p<0.02$, 95% CI for difference 0.3 to 1.5 min; Figure 9.1). The chronotropic responsiveness during upright bicycle exercise was still higher than at baseline (56±4 vs 51±3 bpm, $p=0.11$; -10.0 to 0.04) and the plasma NA lower ($p=0.1$; 95% CI for difference -0.69 to 0.08, Figure 9.5). The power spectral measures of HRV remained significantly different from baseline, with the index of sympathetic activity (LF/HF ratio) remaining significantly lower (Figure 9.4). There were no significant differences in resting or submaximal heart rates, either during incremental dobutamine doses or during upright bicycle exercise, between baseline and 6 weeks following the completion of 3 weeks' PIT with dobutamine.

E. Discussion

This study demonstrates that in patients with severe CHF intermittent short duration PIT produces "pharmacological conditioning" by improving exercise tolerance, autonomic balance and symptoms—changes analogous to the effects of physical training. This "pharmacological conditioning" effect was achieved without concomitant ß-adrenergic receptor down-regulation. On the contrary, PIT up-regulated ß-receptors, thus avoiding one component of tolerance to ß-stimulant therapy.

ß-receptor number may reflect the time course and duration of repeated ß-adrenergic receptor stimulation. It has been found that pharmacological ß-adrenergic stimulation with infusions over 4 hours long produce ß-receptor down-regulation (Tohmeh and Cryer, 1980). This phenomenon parallels the effects of exercise, where ß-receptor activity is reduced when exercise is maintained for longer than 1 hour continuously (Mäki, 1989), whereas shorter bursts of exercise have no effect or actually increase ß-receptor number (Butler *et al.*, 1983; Mäki, 1989). We have confirmed that longer term intermittent pulsed stimulation can increase receptor number so that, if periods of receptor stimulation are kept short enough, down-regulation and drug tolerance can be avoided. The likelihood of dobutamine-induced ventricular arrhythmias is also reduced by keeping the period of infusion shorter.

The increase in lymphocyte ß-receptor numbers was associated with enhanced chronotropic responsiveness, shown by an increased heart rate reserve to both ß-inotropic stimulation and exercise. This finding suggests that the response to sympathetic stimulation is preserved by increased ß-adrenergic receptor responsiveness. The enhancement of ß-adrenoceptor responsiveness may allow lower endogenous catecholamine release and reduced central sympathetic discharge to support the circulation. Sympathetic drive was attenuated in our subjects, as indicated by the reduction in plasma NA concentrations and by the reduction in LF/HF ratio of HRV, an index considered representative of sympathetic activity (Pagani *et al.*, 1986). This beneficial positive feedback could result in an increase in baroreceptor sensitivity as a secondary consequence. Withdrawal of sympathetic tone was first reported with intracoronary infusion of dobutamine in patients with severe CHF (Colucci *et al.*, 1988). A change in basal autonomic tone towards more vagal predominance might be responsible for the reduction in basal heart rate. The significant increase in heart rate difference occurs, however, from submaximal-to-peak exercise test, when the stimulus from the enhanced sympathetic activity and thus the circulating catecholamines is predominant, indicating increased ß-pathway responsiveness rather than a resetting of basal heart rate. Similarly, the increase in heart rate difference becomes significant during the period submaximal-to-peak dobutamine infusion rate, when again the ß-activity of dobutamine is much more prominent.

Chronic activation of the adrenergic system, decreased parasympathetic activity and impaired baroreflex sensitivity have all been described in CHF (Olivari *et al.*, 1983; Eckberg

et al., 1997). We have shown (Coats *et al.*, 1992a) that non-pharmacological approaches, such as physical training, reduce sympathetic activity and increase HRV, both of which are prognostically important abnormalities in CHF (Cohn *et al.*, 1984; Kleiger *et al.*, 1987). This new approach may be a pharmacological way of achieving similar benefits.

Previous studies with long-term treatment with ß-agonists or phosphodiesterase inhibitors have not only failed to improve symptoms and exercise tolerance but also suggested that these agents may accelerate the progression of the underlying disease and provoke the development of complex ventricular arrhythmias and therefore shorten the survival of patients with CHF (Packer and Leier, 1987; Uretsky *et al.*, 1990; Packer *et al.*, 1991). We saw no clinically important arrhythmias in our study. To our knowledge we are the first to report a pharmacological approach using ß-receptor stimulants to improve autonomic function whilst simultaneously up-regulating ß-adrenoceptors. It is possible that restoration of the impaired autonomic balance with PIT may have contributed to the absence of arrhythmic events throughout the study, although the much shorter (than previous studies) infusion periods with dobutamine are obviously also a factor.

Our findings may reflect beneficial changes in central autonomic drive and/or increased baroreflex and cardiopulmonary reflex gain. However, the possibility of an important peripheral action of dobutamine, by improving skeletal muscle ergo-receptor responses and thereby reducing reflex sympathetic activity, cannot be excluded. Improved skeletal muscle blood flow (as a result of the reduction in peripheral vascular resistance) and/or improved skeletal muscle oxidative capacity may reduce the reflex increase in sympathetic tone mediated by the work-sensitive skeletal muscle "ergoreceptors" (metaboreceptors) (Piepoli *et al.*, 1995). Thus, dobutamine-induced $ß_2$-adrenoceptor stimulation resulting in systemic vasodilatation could explain the trend towards reduced peripheral vascular resistance we found. We have previously shown, using ^{31}P MRS, that physical conditioning produces major beneficial alterations in skeletal muscle biochemistry (Adamopoulos *et al.*, 1993) and these may be mimicked by pharmacological conditioning. The importance of peripheral mechanisms to explain the long-term clinical benefits of dobutamine infusions is supported in a 3-week bed rest deconditioning study, where 2 hours of dobutamine daily in normal subjects maintained maximal exercise capacity, aerobic enzyme activity of skeletal muscle, haemodynamic responses and blood volume (Sullivan *et al.*, 1985).

The increased stroke volume we found after the dobutamine infusion period is consistent with the training response in normal subjects (Blomqvist, 1983) and patients with moderate to severe CHF (Leier *et al.*, 1982). It may either represent enhanced diastolic recoil, thus accelerating LV isovolumic relaxation (Parker *et al.*, 1991) and/or a true increase in LV mass and contractile performance. Pulsed inotrope therapy up-regulates the ß-receptor density and so ß-adrenergic receptor stimulation results in an increased production of c-AMP, which plays an important role in regulating LV relaxation.

Erlemeier *et al.* (1992) have reported an increase in exercise tolerance and exercise-provoked chronotropic responsiveness with intermittent 24-hour infusions of dobutamine in humans every 3 days. The absence of drug tolerance was not confirmed by measurement of

ß-receptor activity, which has been shown to be reduced after 4-hour continuous infusion of ß-adrenergic agonists (Tohmeh and Cryer, 1980). Our study confirms these observations but also shows improvement in sympatho-vagal balance and ß-receptor up-regulation.

Liang *et al.* (1979) found remarkable similarities between exercise conditioning and 5-week intermittent dobutamine infusion (2 hours per day) in deconditioned dogs. However, our study is the first to report in humans the same haemodynamic and autonomic effects of PIT accompanied by significant increases in lymphocyte ß-receptor number. Previous experimental studies have also shown that dobutamine improves mechanometabolic efficiency in moderate CHF but depresses mitochondrial function in cardiomyopathic hamsters with advanced heart failure, without improvement in cardiac performance (Buser *et al.*, 1989). This experimental finding is in keeping with previous clinical studies showing that patients with dilated cardiomyopathy in an advanced stage respond poorly to dobutamine (Borow *et al.*, 1988). However, after intermittent dobutamine infusions, degenerative alterations of myocardial mitochondria improved and the mitochondrial number increased (Unverferth *et al.*, 1980). Similarly, in our study short duration PIT may give adequate time for the myocardium to recover after each bout of stimulation.

Finally, our study examined the long-term effects of PIT, indicating persistence, at least partial, of the beneficial effects on autonomic function and exercise capacity for 6 weeks after the completion of the pharmacological conditioning. In a randomised-controlled study looking at the effects of a 72-hour inotrope infusions in advanced CHF the dobutamine group experienced an augmentation in exercise tolerance and ejection fraction lasting for 4 weeks after the infusion period (Liang *et al.*, 1984). However, autonomic balance and tolerance to the inotropic stimulation (expressed by ß-adrenoceptor density down-regulation) characterising long-term continuous inotrope infusions were not described.

With the general availability of ACE inhibitors and vasodilating ß-blockers and with the wide acceptance of the beneficial role of physical training, intermittent inotropic infusions are rarely, if ever, indicated in the long-term management of functional class I, II or III heart failure patients. Pulsed inotrope therapy may, therefore, be useful in patients with CHF who are too severely limited to train (i.e. class IV New York Heart Association, and CHF patients with other chronic illnesses or skeletal myopathies). Short duration PIT along with our growing experience with the proper application of current heart failure therapies may create less "dobutamine-dependent" patients of functional class IV and, therefore, eliminate the need for maintenance continuous inotropic infusions (Leier and Binkley, 1998). Careful selection and monitoring would obviously be essential.

i. Limitations of the Study

Does lymphocyte $ß_2$-receptor density reflect cardiomyocyte $ß_1$-adrenergic receptor regulation? Lymphocytes are exposed only to circulating catecholamines, whereas the heart is affected primarily by innervation. It has been shown, however, that changes in ß-adrenoceptors measured in circulating lymphocytes mirror changes in the density and functional responsiveness of ß-adrenoceptors in the human heart (Brodde *et al.*, 1986).

Although it is possible to show disparity between lymphocyte β_2-receptor number and specific β_1-receptor number on the heart, this disparity is clinically important only when a β_1-selective agonist or antagonist has been used. When a non-selective β-stimulant or blocking agent has been used, the two sub-types of β-adrenoceptors would be expected to be up-regulated or down-regulated in parallel. Both lymphocyte β_2-adrenergic receptors and cardiac β_1-receptors are reduced in congestive heart failure (Colucci *et al.*, 1981; Fowler *et al.*, 1986). A good correlation has been demonstrated between lymphocyte β_2-receptors or cardiac β-adrenoceptors and the severity of CHF (Fowler *et al.*, 1985). There is also good correlation between lymphocyte and cardiac myocyte receptor/second messenger coupling protein (Gs protein) in CHF (Horn *et al.*, 1988). In our study the reduction in arterial NA concentration parallels the reduction in LF component of HRV and the increase in β-receptor density. All these parameters show a similar trend towards reversing the de-coupling of nerve traffic measures (NA concentration) from end-organ response measures (heart rate and HRV measures) observed in CHF. Also the aetiology of CHF may play a role as ischaemic cardiomyopathies exhibit less marked total β- and β_1-receptor down-regulation and a greater degree of uncoupling of left ventricular β_2-receptors compared to idiopathic dilated cardiomyopathies (Bristow *et al.*, 1991). Since most of our studied patients had CHF of ischaemic origin the possibility of having investigated a group with selective down-regulation of β_1 versus β_2-receptors does not seem likely. Our stimulation was generated by a non-specific β-adrenoceptor stimulant on a long-term basis; we can, therefore, postulate that changes in lymphocyte β-receptor number in our study indicate changes in cardiac receptor function.

The clinical results of our trial should be confirmed in a larger group of subjects with both more mild and even more severe heart failure before it could be established that this is definitely a beneficial treatment option in moderate to severe heart failure. The results are, however, encouraging and raise the possibility that β-stimulant agents may be therapeutically important. As a group, however, they may be agents in which chronic day-long administration may not be ideal. Keeping infusions short and pulsing them in the way we have demonstrated, may produce beneficial effects without detrimental receptor down-regulation, although a randomised study of intermittent short versus long administration might be needed.

Chapter X

The Time Course of Haemodynamic, Autonomic and Skeletal Muscle Metabolic Abnormalities Following First Extensive Myocardial Infarction in Man

A. Abstract

Objectives. We sought to investigate the time course of genesis of skeletal muscle dysfunction and sympatho-vagal imbalance after myocardial infarction.

Methods. We studied 22 normal controls, 22 patients with >6 months stable CHF and 10 patients after a first massive myocardial infarction at 1-3 weeks (the 'early' period), 6-8 weeks ('mid') and 6-9 months ('late') following their infarct. Four patients developed overt heart failure. Forearm muscle metabolism was studied using ^{31}phosphorus magnetic resonance spectroscopy (^{31}P MRS). Sympatho-vagal balance was assessed by HRV and radiolabeled NA kinetics.

Results. Increased NA spillover (0.55±0.02 vs 0.27±0.04 mg/min/m^2, $p<0.01$) and decreased HRV, were confined to those post-myocardial infarction patients who subsequently developed heart failure. Resting cardiac output was normal in all the post-myocardial infarction patients, although the response of cardiac output to supine bicycle exercise at the 'mid' study was less in the group who subsequently developed heart failure (9±1% vs 41±8%, $p<0.005$). In the MRS studies, there were no detectable differences between those who did or did not develop heart failure. The initial rate of ATP turnover, calculated from initial-exercise changes in pH and phosphocreatine (PCr), was increased in established CHF, but in the post-myocardial infarction patients a numerically similar increase reached statistical significance only in the early group (19±3 vs 11±1 mM/min, $p<0.005$). The apparent maximum rate of oxidative ATP synthesis, calculated from post-exercise PCr recovery kinetics, was lower than control in late post-myocardial infarction and established CHF groups (34±5 vs 55±4 mM/min, $p<0.03$ and 38±3 vs 55±4 mM/min, $p<0.003$ respectively).

Conclusions. Skeletal muscle metabolism and autonomic function become abnormal after an extensive myocardial infarction. While skeletal muscle abnormalities are relatively slow to develop and unrelated to the degree of failure, excessive neurohormonal activation and impaired cardiac output response to exercise seem from an early stage to characterise patients who subsequently develop CHF.

B. Introduction

Although the underlying mechanisms remain unknown, many pathological changes develop in non-cardiac tissues in the syndrome of CHF. These include reduced peripheral blood flow (Wilson *et al.*, 1984a; Wiener *et al.*, 1986), skeletal muscle atrophy and intrinsic metabolic abnormalities (Massie *et al.*, 1987b; Sullivan *et al.*, 1990; Arnolda *et al.*, 1991; Mancini *et al.*, 1992b; Thompson *et al.*, 1994) and neurohormonal overactivity and autonomic dysfunction (Cohn *et al.*, 1984; Leimbach *et al.*, 1986; Lee and Packer, 1986; Saul *et al.*, 1988; Gottlieb *et al.*, 1989; Osterziel *et al.*, 1995). The progression of left ventricular dysfunction to overt heart failure following acute extensive anterior myocardial infarction is a useful model to study the nature and time course of these pathophysiological sequelae. Neurohormonal hyperactivation and impaired baroreceptor sensitivity have been described during the asymptomatic period preceding overt CHF (Eckberg *et al.*, 1971; La Rovere *et al.*, 1988; Francis *et al.*, 1990a) and the peak aerobic capacity of patients with asymptomatic left ventricular dysfunction is substantially reduced (LeJemtel *et al.*, 1994). However, skeletal muscle metabolic changes in the pre-CHF period following acute myocardial infarction have been demonstrated only in rats (Thompson *et al.*, 1994). Standard treatment trials in established CHF cannot differentiate between specific tissue improvements and changes secondary to alterations in haemodynamics or exercise activities. Studying the time course of changes in central haemodynamics, exercise performance, autonomic balance and skeletal muscle metabolism may help determine which abnormalities are primary and which are secondary to the development of established CHF. This may help in designing strategies for their prevention. We, therefore, investigated prospectively the time course of changes in central haemodynamics (by Doppler ultrasound), exercise performance (by cardiopulmonary exercise test), autonomic balance (by NA spillover and HRV) and skeletal muscle metabolism (by ^{31}P MRS) in 10 patients presenting extensive anterior myocardial infarction as the first manifestation of heart disease. We compared their results to two control groups, one consisting of patients with established CHF and one of normal subjects.

C. Methods

1. Study Population

The investigation conforms with the principles outlined in the Declaration of Helsinki. Inclusion criteria for the post-myocardial infarction patients were: first extensive anterior myocardial infarction (Q-waves in leads V_1-$V_{5,6}$); stable sinus rhythm; absence of symptomatic post-infarct angina or ECG evidence of reversible ischaemia; absence of diabetes mellitus or hypertension; radionuclide ejection fraction ≤40%; and age <70 years. All but 2 patients required diuretics in the coronary care unit and only two patients received ß-blockers in coronary care unit. Ten patients were studied in all (eight men, 2 women; age

42-69, mean 61 years). Measurements were made at 1-3 weeks ('early'), 6-8 weeks ('mid') and 6-9 months ('late') post myocardial infarction.

The patients were retrospectively subdivided into those who developed symptomatic heart failure, defined as symptomatic exercise limitation with documented peripheral or pulmonary oedema at some stage after discharge from hospital (myocardial infarction-heart failure subgroup: 4 patients, of which 3 developed heart failure after the second visit, i.e. after 2 months), and those whose left ventricular dysfunction remained asymptomatic (myocardial infarction-left ventricular dysfunction subgroup: 6 patients). There were no important differences in pharmacological treatment during the early stages after the myocardial infarction between these groups. Three patients were discharged on diuretics (2 of whom later developed heart failure), 3 patients on ß-blockers (1 of whom later developed heart failure) and 8 patients on ACE inhibitors (3 of whom later developed heart failure). Most of the patients were on ACE inhibitors at the late study (9 of 10), while only 5 were taking diuretics and only 3 patients were taking ß-blockers.

For comparison, studies were also performed on 22 patients (one woman, 21 men) with chronic stable heart failure, aged 42-78 (mean 58) years. In these patients the causes of heart failure were coronary artery disease (16 patients), dilated cardiomyopathy (5 patients) and valvular heart disease (one patient). All patients were on diuretics, 17 were also receiving ACE inhibitors, 3 were taking amiodarone, 3 were taking digoxin and 2 were taking ß-blockers. All were in stable CHF with no recent (less than 6 months) myocardial infarction or coronary artery bypass grafting or change in medications. Twenty-two healthy controls of similar age (37-77, mean 54 years; 5 women, 17 men) were studied using MRS; for non-MRS measurements, we studied 12 different control subjects of similar age-range (39-66, mean 53).

ii. Magnetic Resonance Spectroscopy Methods

The dominant arm was placed in a 1.9 T superconducting magnet (Oxford Instruments, Oxford, U.K.) interfaced to a Biospec spectrometer (Oxford Research Systems, Oxford, U.K.) and a 2.5 cm diameter surface coil was placed over the muscle. Spectra were acquired using a 2-sec interpulse delay at rest (128 scans) and during finger flexion (32 scans) at a power output of 0.25-W for four spectra, incremented by 0.08-W for each of the remaining spectra. Exercise was continued until exhaustion. The muscle was then studied for 12 min during recovery (four 16-scan spectra, four 32-scan spectra and then two 64-scan spectra) (Stratton *et al.*, 1994).

Relative concentrations of inorganic phosphate (Pi), PCr and ATP were obtained by manual triangulation of peak areas and were corrected for saturation. Absolute concentrations were obtained assuming a normal ATP concentration of 8.2 mM (Arnold *et al.*, 1984). During exercise it is convenient to express PCr concentration as PCr/(PCr+Pi) to allow for possible changes in signal intensity due to movement. Intracellular pH was calculated from the chemical shift of the Pi peak, relative to PCr (Arnold *et al.*, 1984). Free cytosolic adenosine diphosphate (ADP) concentration was calculated from pH and PCr

concentration using the creatine kinase equilibrium constant (Veech *et al.*, 1979) of K_{eq} = $1.66x10^9$ M^{-1}, assuming a normal total creatine content (Arnold *et al.*, 1984) of 42.5 mM. Note that there was no significant alteration in concentrations of ATP or total creatine in a rat model of severe heart failure (Arnolda *et al.*, 1991; Thompson *et al.*, 1994).

The details of the analysis of the MRS data from exercise and recovery have been published elsewhere (Kemp *et al.*, 1994a) and are briefly outlined here. During exercise, ATP is produced by net hydrolysis of PCr, by glycogenolysis to lactic acid and by oxidative phosphorylation; protons are produced by glycogenolysis, buffered both by passive processes and as a consequence of PCr breakdown, and also leave the cell by several processes. Thus, changes in pH, PCr and ADP during exercise depend on contractile efficiency and power output (which determine total ATP turnover), on glycogenolytic and oxidative ATP synthesis, and on net proton efflux from the cell (Kemp *et al.*, 1993b and 1994).

In early exercise, oxidative ATP synthesis and proton efflux make little contribution (Kemp *et al.*, 1994b) so comparison of the response at the initial 'plateau', before power output is made to increment, reflects only total ATP demand, oxidative function and the resulting need for non-oxidative ATP synthesis. Furthermore, measurements of pH and PCr concentration from rest to the first exercise spectrum can be used to calculate the initial rate of non-oxidative ATP synthesis (Kemp *et al.*, 1994b), which is inversely proportional to muscle mass (cross-sectional area) and to contractile efficiency (work done per ATP hydrolysed).

Subjects exercised to exhaustion, and so end-exercise values of measured variables depend also on susceptibility to fatigue, which complicates their interpretation. End-exercise state influences recovery from exercise, during which PCr is resynthesised as a consequence of oxidative ATP synthesis. The initial rate of PCr resynthesis is an estimate of the end-exercise rate of oxidative ATP synthesis and has a hyperbolic (Michaelis-Menten) relationship to its driving force, the end-exercise cytosolic ADP concentration (Kemp *et al.*, 1993a). These are used to calculate the apparent maximum rate of oxidative ATP synthesis (Q_{max}), which is a quantitative measure of mitochondrial content, mitochondrial 'activation state' and blood flow (Kemp *et al.*, 1993c).

iii. Autonomic Balance Methods

Autonomic function was assessed by analysis of HRV and measurement of radiolabeled NA kinetics: i) Heart rate variability was measured from 640 consecutive beats (lead V_5) recorded in a quiet darkened environment after 30 min daytime bed rest using a Store 4 Racal-Thermionic FM tape recorder (Southampton, UK). Heart rate variability was measured in the time domain by the standard deviation of those R-R intervals having normal morphology and a cycle length within 80-120% of the preceding cycle length. Heart rate variability was also measured in the frequency domain using power spectral analysis, performed using an autoregressive model (Kay and Marple, 1981; Coats *et al.*, 1992a), which

evaluates the power of harmonic components in the ranges 0.03-0.14 Hz (LF component, predominantly representing sympathetic tone) and 0.18-0.40 Hz (HF component, predominantly representing vagal activity) (Pomeranz *et al.*, 1985; Pagani *et al.*, 1986), expressed as a percentage of the total oscillatory power (normalised units, nu). The LF/HF ratio was also calculated and taken as an index of sympathetic activity (Pomeranz *et al.*, 1985; Pagani *et al.*, 1986). ii) Noradrenaline kinetics were measured using a standard methodology, which is described in detail elsewhere (Esler *et al.*, 1979). The normal range of plasma NA in our laboratory is 120-300 pg/ml.

iv. Exercise Testing and Haemodynamic Measurements

Exercise tests were performed in the fasted state before daily medication, using a Tunturi Professional Ergometer (Tunturi, Finland). The upright bicycle tests were performed in 5-min stages, incrementing by 25-W to the limit of tolerance, with 1-min average measurements of oxygen consumption and CO_2 production using standard methods (Coats *et al.*, 1990). After 1-hour rest, a similar, but supine, exercise was performed during which haemodynamic measurements were made at rest and at the end of each increment (Elema-Schonander supine electrically braked cycle ergometer, AM 368, Stockholm, Sweden). Pulsed wave Doppler ultrasonic measurements of ascending aortic blood velocity from the suprasternal approach were made using a Pedof Doppler ultrasound generator (Vingmed, Norway) and our own laboratory-made Fast Fourier Transform spectral analyser (Murphy *et al.*, 1988). Using the intensity-weighted mean frequency in each 5-msec time bin, stroke distance was calculated as the integral of velocity and time for the ejection period. Using standard formulae and an echocardiographic measurement of aortic cross-sectional area (leading edge to leading edge, immediately distal to the sinus of Valsalva), stroke volume was calculated by a previously-validated method in our laboratory (Coats *et al.*, 1992b). Blood pressure was measured using a previously-validated automatic sphygmomanometer (Copal UA 251, Takeda Medical, Tokyo) (Coats *et al.*, 1989).

v. Statistical Analysis

Measurements in the three post-myocardial infarction studies were compared by analysis of variance for repeated measures, followed by post-hoc Mann-Whitney U tests. Measurements in the CHF and control groups were compared by Mann-Whitney U test. Results at $p<0.05$ are taken as significant (corrected where appropriate for multiple comparison testing by the Scheffe procedure). Data are presented as mean±SEM.

D. Results

i. Post-Myocardial Infarction Patients Compared to Controls and Patients with Established Heart Failure

Table 10.1 shows data on haemodynamic and autonomic function at the early, mid and late post-myocardial infarction periods. The pattern was essentially similar for ejection fraction, for HRV measured by time domain or frequency domain methods, for plasma NA and NA spillover, and also for peak oxygen uptake and cardiac output at 50-W. For all these measurements (except that no early data were available for cardiac output and oxygen uptake), no difference was detected between the early, mid and late post-myocardial infarction studies, but all of these were abnormal with respect to controls. The established CHF group showed similar abnormalities in comparison to controls. Only resting cardiac output and resting heart rate showed no significant abnormalities in either post-myocardial infarction or established CHF groups.

Table 10.2 shows data from MRS studies. There were no significant abnormalities in muscle at rest except a low PCr/(PCr+Pi) in the late post-myocardial infarction group. There were no significant abnormalities in measurements taken at the end of exercise. Exercise duration, however, was significantly reduced in the early and mid

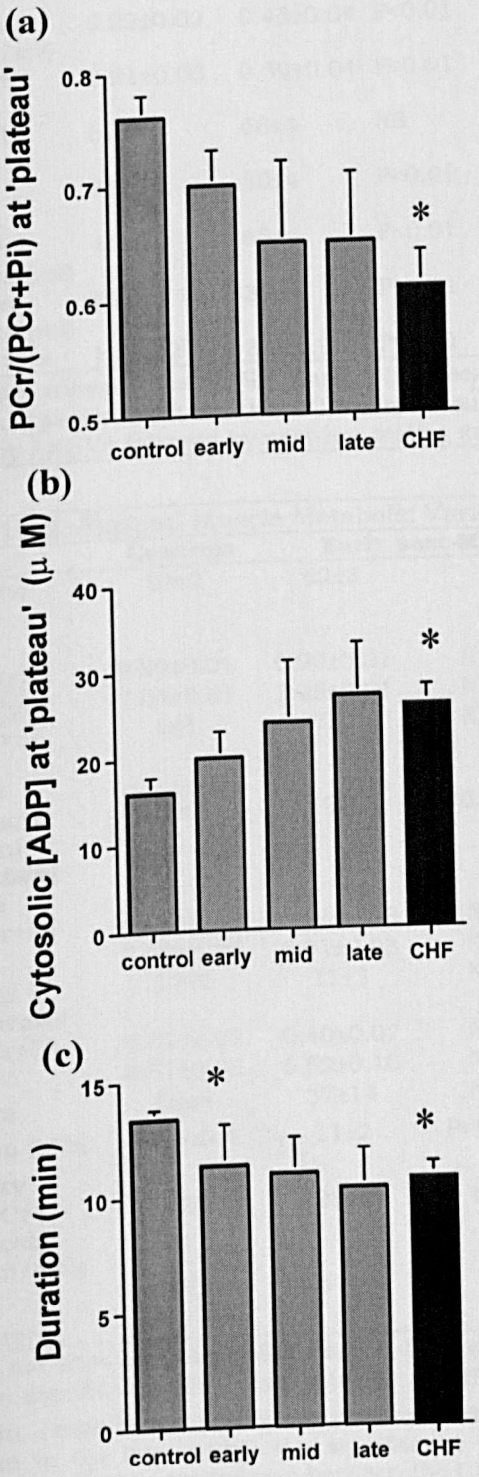

Figure 10.1. Time course of muscle metabolic response after a large myocardial infarction. Metabolic changes during exercise in *flexor digitorum superficialis*. The figure shows (a) phosphocreatine (PCr) concentration, expressed as PCr/(PCr+Pi), (b) adenosine diphosphate (ADP) concentration measured at the submaximal exercise plateau, and (c) exercise duration. Results are shown for control subjects, patients following first extensive anterior myocardial infarction at early (1-4 weeks), mid (6-8 weeks) and late (6-9 months) intervals, and patients with established chronic heart failure. Asterisks denote statistically significant difference ($p<0.05$) compared to the normal controls. Results are shown as mean±SEM.

TABLE 10.1. Haemodynamic and Autonomic Variables in Post-MI Patients Compared to Control Subjects, and in Patients with Established CHF

	Controls	Early post-MI		Mid post-MI		Late post-MI		Established CHF	
EF (%)	56±2	21±3	P<0.001	21±2	P<0.001	25±3	P<0.001	25±2	P<0.001
VO_2 max (ml/kg/min)	26±2	-	-	14±1	P<0.001	12±2	P<0.001	12±1	P<0.001
Resting CO (l/min)	5.3±0.4	4.5±0.7	NS	4.4±0.8	NS	4.3±0.7	NS	4.7±0.4	NS
50-W CO (l/min)	8.4±0.7	-	-	5.4±0.9	P<0.001	5.3±0.8	P<0.001	6.1±0.6	P<0.001
NA spillover ($mg/min/m^2$)	0.22±0.02	0.43±0.04	P<0.01	0.37±0.04	P<0.01	0.37±0.05	P<0.01	0.42±0.03	P<0.01
Plasma NA (ng/ml)	0.21±0.03	0.39±0.04	P<0.01	0.34±0.03	P<0.01	0.34±0.04	P<0.01	0.37±0.03	P<0.01
Heart rate (bpm)	69±5	68±4	NS	72±5	NS	71±5	NS	78±4	NS
SD -RR (msec)	55±5	30±4	P<0.01	33±4	P<0.01	29±5	P<0.01	28±4	P<0.01
LF-power spectrum (nu)	44±5	65±4	P<0.01	57±5	NS	59±5	P<0.01	66±4	P<0.01
HF-power spectrum (nu)	50±5	30±4	P<0.01	36±5	P<0.01	34±5	P<0.01	26±4	P<0.01
LF/HF ratio	1.0±0.2	3.2±0.9	P<0.01	2.2±0.5	P<0.05	2.4±0.6	P<0.05	4.0±0.9	P<0.001

Results are shown as mean±SE. Early = 1-3 weeks post-MI; mid = 6-8 weeks post-MI; late = 6-9 months post-MI; VO_2 max = peak oxygen uptake; CO = cardiac output; NA = noradrenaline; *P* = significance of difference from control by ANOVA followed by post-hoc testing and between the CHF group and control by Mann-Whitney U test

TABLE 10.2. Skeletal Muscle Metabolic Variables in Post-MI Patients

	Controls	Early post-MI		Mid post-MI		Late post-MI		Established CHF	
Age (years)	54±2	62±3						58±2	
Resting muscle									
PCr/(PCr+Pi)	0.89±0.01	0.90±0.01	NS	0.90±0.01	NS	0.87±0.01	P<0.03	0.89±0.01	NS
pH	7.04±0.01	7.05±0.01	NS	7.05±0.01	NS	7.01±0.01	NS	7.01±0.01	NS
[ADP] μm	6±1	7±2	NS	7±2	NS	8±2	NS	7±1	NS
Initial exercise									
ATP turnover (mm/min)	11±1	19±3	P<0.005	19±5	NS	17±5	NS	21±2	P<0.0005
Submaximal exercise									
PCr/(PCr+Pi)	0.75±0.02	0.71±0.03	NS	0.65±0.07	NS	0.68±0.09	NS	0.63±0.03	P<0.01
pH	7.00±0.02	6.91±0.03	NS	6.89±0.08	NS	7.02±0.06	NS	6.85±0.04	P<0.002
[ADP] μm	17±2	17±3	NS	20±4	NS	24±8	NS	23±2	P<0.05
End exercise									
PCr/(PCr+Pi)	0.37±0.03	0.40±0.07	NS	0.43±0.07	NS	0.37±0.09	NS	0.35±0.03	NS
PH	6.51±0.05	6.52±0.10	NS	6.52±0.09	NS	6.74±0.06	NS	6.51±0.06	NS
[ADP] μm	32±4	37±14	NS	28±6	NS	72±27	NS	40±6	NS
Duration (min)	13.4±0.4	11±2	P<0.02	12±2	NS	10±2	P=0.06	11±1	P<0.002
Recovery									
Initial PCr resynthesis rate (mm/min)	25±2	28±4	NS	21±2	NS	22±5	NS	20±2	NS
Qmax (mm/min)	55±4	64±12	NS	50±7	NS	34±5	p<0.03	38±3	P<0.003

Results are shown as mean±SE. Early = 1-3 weeks post-MI; mid = 6-8 weeks post-MI; late = 6-9 months post-MI. There were no significant differences between MI-HF group and MI-LVD group (e.g. initial ATP turnover = 24±7 vs. 16±1 mM/min, respectively, in the early study; duration = 10±2 vs. 12±2 min in the mid study; Qmax = 42±12 vs. 35±5 mM/min in the late study). *P* = significance of difference between the whole post-MI group and control by ANOVA followed by post-hoc testing and between the CHF group and control by Mann-Whitney U test.

post-myocardial infarction studies and in the established CHF group. At 'submaximal' exercise (i.e. the stable plateau before muscle power output was incremented), there were no significant abnormalities in the post-myocardial infarction patients at any stage, although in the established CHF group PCr/(PCr+Pi) and pH were lower and ADP higher than in controls (Figure 10.1). The initial rate of ATP turnover (Figure 10.2a) was significantly abnormal in the established CHF group, being nearly twice as large as in controls. In the post-myocardial infarction studies the rate was numerically similar to the established CHF value, but the difference reached statistical significance only in the early study (presumably due to the relatively small sample size). As calculated from the recovery kinetics, the apparent maximum rate of oxidative ATP synthesis (Q_{max}) was significantly lower than control in late post-myocardial infarction study and in the established CHF group (Figure 10.2b).

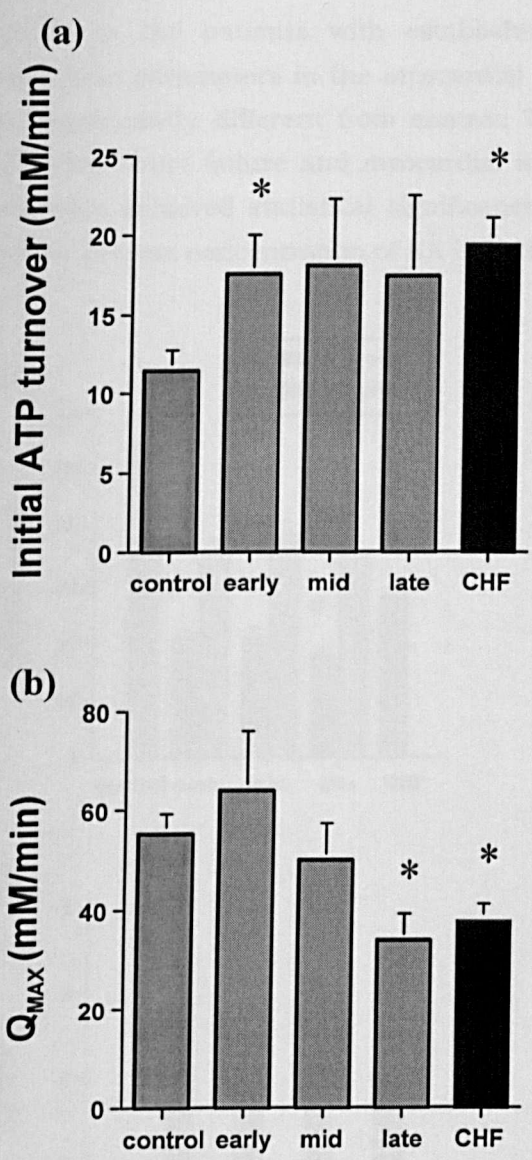

Figure 10.2. Time course of muscle metabolic response after a large myocardial infarction. Metabolic quantities calculated from MRS data in *flexor digitorum superficialis*. The figure shows (a) the initial rate of ATP turnover, and (b) the apparent maximum rate of mitochondrial ATP synthesis. Results are shown for control subjects, patients following first extensive anterior myocardial infarction at early (1-4 weeks), mid (6-8 weeks) and late (6-9 months) intervals, and patients with established chronic heart failure. Asterisks denote statistically significant difference ($p<0.05$) compared to the normal controls. Results are shown as mean±SEM.

ii. Comparison Between Post-Myocardial Infarction Patients Who Do and Do not Develop Heart Failure

A surprising difference in autonomic function became apparent when the patients were retrospectively divided into those who went on to develop heart failure (the myocardial infarction-heart failure group) and those who did not (the myocardial infarction-left ventricular dysfunction group). In all three studies following myocardial infarction the myocardial infarction-heart failure subgroup showed significantly higher heart rate, lower SDRR, higher LF and lower HF components (and therefore

higher LF/HF ratio) than the myocardial infarction-left ventricular dysfunction group (Table 10.3). For these measurements the myocardial infarction-heart failure group showed values similar to the patients with established CHF (Figsures 10.3a & 10.3b), while these autonomic parameters in the myocardial infarction-left ventricular dysfunction group were not significantly different from control. This implies a difference between the myocardial infarction-heart failure and myocardial infarction-left ventricular dysfunction groups: this difference achieved statistical significance for NA spillover at the late study (Figure 10.3c) and for plasma concentration of NA in both the mid and late studies.

The situation was different with haemodynamic and oxygen consumption measurements. The myocardial infarction-heart failure subgroup had a significantly lower peak oxygen uptake than myocardial infarction-left ventricular dysfunction in both the mid and late studies, although values for both subgroups were lower than control. The ejection fraction, in contrast, did not differ between myocardial infarction-heart failure and myocardial infarction-left ventricular dysfunction subgroups in any of the post-myocardial infarction studies, being lower than control in all subgroups. Resting cardiac output was normal in both the myocardial infarction-heart failure and myocardial infarction-left ventricular dysfunction subgroups (Table 10.3). However, the response of cardiac output to supine bicycle exercise in the mid study was less in the myocardial infarction-heart failure

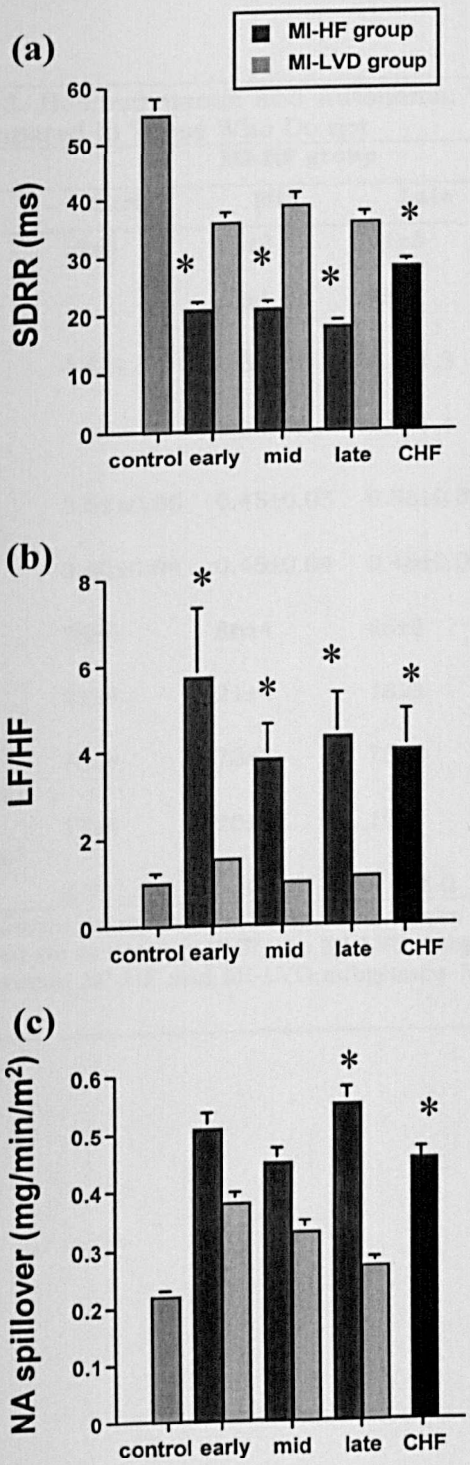

Figure 10.3. Time course of sympatho-vagal balance after a large myocardial infarction. The figure shows (A) standard deviation of all normal R-R intervals, (B) ratio of low-frequency to high-frequency components (LF/HF) in the power spectrum of the heart rate, and (C) noradrenaline (NA) spillover in control subjects, in patients following first extensive anterior myocardial infarction at early (1-4 weeks), mid (6-8 weeks) and late (6-9 months) intervals, and in patients with established chronic heart failure. For the post-myocardial infarction patients, data are divided between patients who subsequently develop chronic heart failure (the myocardial infarction-heart failure subgroup, MI-HF) and those who did not (the myocardial infarction-left ventricular dysfunction subgroup, MI-LVD). Asterisks denote statistically significant difference between the myocardial infarction-heart failure and myocardial infarction-left ventricular dysfunction subgroups. Results are shown as mean± SEM.

group than in myocardial infarction-left ventricular dysfunction (per cent increase in cardiac output from rest to 50-W: 9±1% vs 41±8%, p<0.005).

The situation was different again for MRS measurements, where (in contrast to these findings) no significant difference could be demonstrated between the myocardial infarction-heart failure and myocardial infarction-left ventricular dysfunction groups. Thus, the myocardial infarction-heart failure group showed no difference in rest or 'submaximal' exercise pH, PCr/(PCr+Pi) and ADP concentration, in initial rate of ATP turnover and in apparent maximum rate of oxidative ATP synthesis in all three periods following myocardial infarction compared to myocardial infarction-left ventricular dysfunction group.

TABLE 10.3. Haemodynamic and Autonomic Variables in Post-MI Patients: Those that Go on to Develop Heart Failure Compared to Those Who Do not

	MI-HF group			MI-LVD group					
	Early	**Mid**	**Late**	**Early**		**Mid**		**Late**	
EF (%)	19±3	18±2	21±3	24±5	NS	23±3	NS	29±4	NS
VO_2 max (ml/kg/min)	-	10±1	9±1	-	-	16±1	P<0.01	16±1	P<0.01
Resting CO (l/min)	4.6±1.3	4.6±1.3	4.5±1.3	4.4±0.9	NS	4.3±1.0	NS	4.2±0.8	NS
50-W CO (l/min)	-	5.0±1.4	4.9±1.4	-	-	5.8±1.2	NS	5.8±1.0	NS
NA spillover (mg/min/m^2)	0.51±0.05	0.45±0.05	0.55±0.02	0.38±0.04	NS	0.33±0.05	NS	0.27±0.04	P<0.01
Plasma NA (ng/ml)	0.46±0.04	0.45±0.04	0.46±0.06	0.34±0.05	P<0.001	0.27±0.01	P<0.001	0.26±0.02	P<0.001
Heart rate (bpm)	76±5	86±4	86±3	62±4	P<0.05	62±4	P<0.005	61±4	P<0.005
SD -RR (msec)	21±4	21±4	18±3	36±4	P<0.05	39±5	P<0.01	36±6	P<0.05
LF-power spectrum (nu)	78±4	73±5	75±3	57±2	P<0.005	46±3	P<0.005	48±2	P<0.001
HF-power spectrum (nu)	17±4	20±2	19±3	38±2	P<0.001	47±4	P<0.005	44±3	P<0.001
LF/HF ratio	5.7±1.6	3.8±0.8	4.3±1.0	1.5±0.1	P<0.01	1.0±0.1	P<0.005	1.1±0.1	P<0.005

Results are shown as mean±SE. Early = 1-3 weeks post-MI; mid = 6-8 weeks post-MI; late = 6-9 months post-MI. The MI-HF subgroup went on to develop CHF, the MI-LVD subgroup had only asymptomatic left ventricle dysfunction. *P* = significance of difference between MI-HF and MI-LVD subgroups. Values in control subjects and in patients with established CHF are given in Table 1.

E. Discussion

We believe this is the first human study to describe the time course of changes in central haemodynamics, functional capacity, autonomic balance and skeletal muscle metabolism following an extensive first anterior myocardial infarction, a condition which is characterised by a high risk of progression to CHF. The value of this model is that we can study patients assuming a relatively normal skeletal muscle function immediately after their first myocardial infarction, through to a period when some have developed stable CHF with its known secondary non-cardiac pathophysiological changes. We will discuss separately the findings for autonomic balance, central haemodynamics and skeletal muscle metabolism.

i. Neurohormonal Activation

Neurohormonal activation early after myocardial injury, although acting initially as a compensatory mechanism, may play an important role in the pathophysiology of the preclinical stage of left ventricular dysfunction by exacerbating the haemodynamic abnormalities and by exerting direct toxic effects on the myocardium (Packer, 1992a). The plasma concentrations of several neurohormones (including NA, atrial natriuretic peptide and plasma renin activity) are elevated in CHF, and are associated with increased mortality rates (Cohn *et al.*, 1984, Lee and Packer, 1986, Gottlieb *et al.*, 1989). It has hitherto been unclear whether this is a late manifestation secondary to heart failure or an early marker that precedes the clinical syndrome. Although plasma NA concentrations are less elevated in asymptomatic left ventricular dysfunction than in clinically overt CHF, even modest elevations in plasma NA predict all-cause and cardiovascular mortality and morbidity (Benedict *et al.*, 1996).

Our study provides a detailed description of the sympatho-vagal balance at three different intervals during the first 9 months following a first and extensive anterior myocardial infarction. It shows that persistent neurohormonal hyperactivation in the post-myocardial infarction period, evident in plasma NA kinetics and in HRV, characterises patients who subsequently develop CHF, and differentiates them from those who, despite similar degrees of left ventricular dysfunction, do not manifest symptoms or signs of CHF.

ii. Haemodynamic Abnormalities

The lower response of cardiac output to exercise found in asymptomatic patients who subsequently manifested CHF indicates an impaired myocardial contractile reserve, which limits peak oxygen consumption in asymptomatic patients with left ventricular dysfunction (LeJemtel *et al.*, 1994). The impaired contractile reserve is contributed to by the defective chronotropic responsiveness to exercise (observed both in asymptomatic left ventricular dysfunction and in overt CHF) (LeJemtel *et al.*, 1994), which results from parasympathetic and sympathetic abnormalities (Goldstein *et al.*, 1975) due, at least partly, to postsynaptic desensitisation of the ß-adrenergic pathway (Colucci *et al.*, 1989). In the present study both

impaired contractile reserve and sympathetic predominance were predictors of the subsequent development of CHF.

The possible confounding influence of the ACE inhibitors, ß-blockers and digitalis should be taken into account. However, almost all of the patients were on ACE inhibitors throughout the study period; ß-blockers and digitalis have been demonstrated to produce early, profound and sustained reduction in sympathetic nerve activity in patients with CHF (Ferguson *et al.*, 1989), but there were only 2 and 1 subjects respectively taking these agents in this study, rendering the number too small to determine their significance in interfering with pathophysiological sequelae following extensive myocardial infarction.

iii. Muscle Metabolic Abnormalities

It has been shown elsewhere (Kemp *et al.*, 1996) that ^{31}P MRS abnormalities in CHF can be explained largely in terms of a decrease in 'effective muscle mass' (which may include changes in actual muscle mass as well as altered contractile efficiency due to e.g. fibre type changes), and a decrease in mitochondrial oxidative capacity (due, for example, to reduced mitochondrial content or to an abnormal blood flow response to exercise). In the present study, in the post-myocardial infarction group as a whole only the late study revealed a significant abnormality in mitochondrial oxidative capacity. While an abnormality in initial ATP turnover (which is inversely related to 'effective muscle mass') was significant only in the early study, the values for all post-myocardial infarction groups were numerically similar to the established CHF group. Figures 10.1 and 10.2 suggest a general trend for the abnormalities in measurements made at submaximal exercise (Figures 10.1a & 10.1b), tolerated exercise duration (Figure 10.1c) and apparent mitochondrial capacity (Figure 10.2b) to increase with time from the early study to the late, although none of these trends reaches statistical significance. We cannot rule out a progressive element, nor prove it, and it is evident that to do either would require a much larger study. Nor can we say for certain that muscle metabolic abnormalities bear no relationsnip to the subsequent development of CHF, although no such difference could be demonstrated here. Nevertheless, it seems unlikely that the muscle abnormalities can be primary events in the pathophysiology of CHF, although the present work shows that some of the abnormalities can develop quite early.

In general, skeletal muscle metabolic abnormalities in CHF could be attributed to reduced cardiac output, to physical deconditioning, or to other effects such as neurohormonal, immunological or catabolic alterations. Some investigators (Wilson *et al.*, 1984b) have reported impaired blood flow during exercise in CHF, which could contribute to the abnormality in mitochondrial ATP synthesis. However, there is evidence from blood flow measurements (Wiener *et al.*, 1986, Massie *et al.*, 1987 a&b), studies during ischaemic exercise (Massie *et al.*, 1988) and responses to localised exercise training (Minotti *et al.*, 1990b) that metabolic abnormalities also occur independent of changes in blood flow. In the present study, the response of cardiac output to exercise was reduced only in patients who subsequently developed congestive heart failure, while muscle metabolic abnormalities were

unrelated to subsequent CHF. Bioenergetic abnormalities, consisting of impaired oxidative phosphorylation, have also been shown in rats following myocardial infarction without haemodynamic compromise, using the more sensitive recovery measurements obtained by ^{31}P MRS studies. These post-infarct changes are similar in extent to the reduction in effective mitochondrial capacity seen in other clinical conditions (Kemp *et al.*, 1993a), implying that at least some of the skeletal muscle metabolic abnormalities seen in heart failure are unrelated to blood flow or to muscle atrophy or to the clinical severity of the cardiac dysfunction and part of the explanation is thought to be a mitochondrial defect.

Finally, although speculative, interpretation of impaired muscle bioenergetics after a first extensive myocardial infarction may emerge from muscle-specific gene expression. Despite the differences in the patterns of expression in muscle-specific genes in the heart and in skeletal muscle cells, injury of myocardial cells may induce regulatory factors to express the same genes in both the heart and skeletal muscle (Sartorelli *et al.*, 1993). Cross talk of muscle-specific gene expression could provide the theoretical basis of interference with skeletal muscle bioenergetics, thus explaining alterations in skeletal muscle metabolism following extensive myocardial infarction.

iv. Clinical Implications

This study offers a more detailed understanding of the integrated pathophysiology underlying both the progression of the disease and the complex interaction between exercise responses and symptoms. This may allow more effective therapeutic strategies to be designed by looking beyond the conventional haemodynamic remedies for heart failure. We have found that myocardial contractile reserve, measured during the asymptomatic period after an extensive myocardial infarction, was impaired in the subgroup of patients who manifested symptoms of CHF a few months later. Skeletal muscle metabolism and autonomic function were also abnormal, although they did not follow similar patterns. Patients who subsequently developed CHF were from an early stage characterised by persistent neurohormonal hyperactivation with sympathetic predominance, clearly distinguishable from those who did not subsequently develop CHF. In contrast, no significant difference between these groups could be demonstrated for muscle metabolism.

Neurohormonal activation early in the process of left ventricular dysfunction plays an important role in the pathogenesis and progress of the syndrome of CHF. According to the muscle hypothesis (Coats *et al.*, 1994), skeletal muscle dysfunction may significantly contribute to the progression of the disease by exaggerating the sympathetic nervous responses to exercise. Early pharmacological and other interventions may therefore modify the natural history of CHF by attenuating sympatho-vagal activation, improving baroreceptor sensitivity and partially reversing intrinsic skeletal muscle metabolic abnormalities.

This is a clinical study and although we cannot control for all the factors, which may impact on the measured variables, we have chosen a fairly homogenous patient sub-set for study and the observations are of a nature that cannot easily be modelled in an animal

model of treatment-related long-term adaptations to the development of heart failure in the human setting. We, therefore, feel that the results would be interest to both a clinical and scientific readership interested in the mechanisms of heart failure-related pathophysiological changes.

v. Limitations of the Study

This study is relatively small due to the strict inclusion criteria. Despite our choice of patients with no symptoms prior to the infarct we cannot exclude a small effect on non-cardiac tissues of prior subclinical coronary disease with left ventricular dysfunction. The fact that skeletal muscle metabolism was relatively normal in the early and mid studies suggests that this was unlikely, at least for skeletal muscle, but it may be important for autonomic function.

Conclusions of the Thesis

Human and animal studies on skeletal muscle and autonomic function in chronic heart failure, with particular emphasis on the role of exercise training, have been discussed and important observations have been emerged.

A Methodological aspects were initially discussed regarding assessment of autonomic balance in patients with chronic heart failure:

I. To quantify the reproducibility of heart rate variability measures (standard deviation of R-R intervals together with low- and high-frequency components of heart rate variability using an autoregressive algorithm) from short-term sampling periods 10 patients with chronic heart failure were evaluated during stable conditions and during two different sympathetic stimulations: physical exercise and inotrope infusion. The low-frequency index showed fairly good reproducibility during sympathetic stimulations, whereas the standard deviation of R-R intervals and the high-frequency indices were slightly less reproducible, particularly at the higher levels of sympathetic stimulation. These relatively moderate day-to-day variations should be considered when heart rate variability determinations are used to assess alterations in the autonomic control of the cardiovascular system, particularly during dynamic conditions.

II. To evaluate the ability of different methods to describe sympatho-vagal balance in chronic heart failure 25 patients with moderate to severe chronic heart failure were studied before and after 8 weeks of physical training at home in a randomised crossover design. After 8 weeks of exercise training all methods (24-hour daytime and nocturnal heart rate, submaximal heart rate during bicycle exercise, heart rate variability measures in the time and frequency domain and radiolabeled noradrenaline spillover) showed improvement in autonomic function. However, neither the absolute values before and after training nor the training-induced percent changes of the individual measures of autonomic function showed a significant correlation between each other. The lack of intermethod correlations suggests that in chronic heart failure the individual parameters of autonomic control may reflect different aspects of circulatory control and, therefore, a comprehensive description of autonomic tone probably requires multiple methods.

III. In our ^{31}P MRS studies of skeletal muscle metabolism in heart failure, recovery kinetics of phosphocreatine (phosphocreatine resynthesis half-time and the initial rate of phosphocreatine resynthesis) were used to estimate mitochondrial oxidative capacity, given that end-exercise values of measured variables (pH, inorganic phosphate, phosphocreatine and adenosine diphosphate concentrations) depend also on susceptibility to muscle fatigue, thus complicating their interpretation. More specifically, the end-exercise adenosine diphosphate concentration and initial phosphocreatine resynthesis rate were used to calculate the *maximum rate of oxidative ATP synthesis* (Q_{max}) which is independent of muscle mass and workload and represents a quantitative measure of mitochondrial content, mitochondrial 'activation state' and blood flow. Furthermore, measurements of pH and

phosphocreatine concentration from rest to the first exercise spectrum were used to calculate the initial rate of non-oxidative ATP synthesis, which is inversely proportional to muscle mass (cross-sectional area) and to contractile efficiency (work done per ATP hydrolysed). The combination of true exercising muscle mass and contractile efficiency was expressed as *effective muscle mass.*

B. In our human and animal studies the role of physical training programmes on skeletal muscle metabolism in experimental and human heart failure was evaluated, the effects of physical training on autonomic balance in stable chronic heart failure were examined, the effects of inotrope 'training' (by pulsing ß-stimulant therapy) on exercise performance, ß-adrenoceptors density, chronotropic responsiveness and autonomic function in patients with chronic heart failure were assessed and finally the time course of central haemodynamics, autonomic function and skeletal muscle metabolism in patients following extensive anterior myocardial infarction was described:

I. The effects of exercise training on skeletal muscle metabolic abnormalities, characterising the complex syndrome of chronic heart failure, were examined in both experimental and human heart failure.

Initially the influence of physical training on skeletal muscle metabolism after myocardial infarction was studied in a rat model of the development of heart failure. Rats with congestive heart failure developed similar skeletal muscle metabolic changes in the handling of high energy phosphates to those described in heart failure in humans. More important was the training-induced normalisation of skeletal muscle metabolism, as reflected by the lower phosphocreatine and pH during sciatic nerve stimulation and by the longer phosphocreatine and adenosine diphosphate recovery half-times only in the non-trained group of animals with congestive heart failure. This normalisation, including correction of the lower citrate synthase activity seen also only in the non-trained rats with congestive heart failure, was achieved without any change in calf muscle mass or individual fibre size between trained and non-trained animals, making, therefore, atrophy unlikely to be the sole cause of the muscle metabolic changes characterising heart failure. Subsequently, the effects of physical training on skeletal muscle metabolism in patients with chronic heart failure were investigated by using ^{31}P magnetic resonance spectroscopy. Physical training in humans produced a significant reduction in phosphocreatine depletion and a blunted rise of adenosine diphosphate concentration during exercise as well as an enhanced rate of phosphocreatine resynthesis during recovery (an index of mitochondrial oxidative capacity which is independent of muscle mass). These findings are compatible with an increased capacity of oxidative ATP synthesis, indicative of an increase in either mitochondrial content or functional capacity of the existing mitochondria.

Exercise training programmes in rats and in patients with congestive heart failure can, therefore, achieve a substantial correction of the impaired oxidative capacity of skeletal muscle, indicating that physical deconditioning contributes to the aetiology of skeletal muscle metabolic abnormalities. In addition, the experimental study suggests that alterations in oxidative capacity of skeletal muscle could provide the biochemical basis of

the magnetic resonance spectroscopy abnormalities seen in both the trained and the untrained state.

II. Physical deconditioning may cause or perpetuate some of the secondary changes observed in chronic heart failure. A study was, therefore, conducted to give some insight into whether exercise training alone can reverse, at least partially, the autonomic features of deconditioning seen in chronic heart failure and thereby improve symptoms and exercise performance.

Physical training produced significant, and perhaps important, reductions in 24-hour heart rate, day and night heart rate, submaximal workload exercise heart rate, markedly increased heart rate variability in the time domain in both waking and sleep states (expressed by standard deviation of normal morphology R-R intervals), significant improvement in heart rate variability in the frequency domain (using power spectral analysis of the resting ECG) consisted of reduction in the low-frequency component and increase in the high-frequency component and significant reduction in whole-body radiolabeled noradrenaline spillover. These measurements all showed an important shift away from sympathetic towards increased vagal activity after training and may reflect beneficial changes in the baroreceptor sensitivity and skeletal muscle ergoreflex.

A well-defined diurnal pattern of dynamic changes in sympatho-vagal balance has been recently linked with the circadian variation of acute cardiovascular events. The circadian variations of the low- and high-frequency components of heart rate variability and the effect of exercise training on the circadian pattern of heart rate variability, recorded over 24 hours in relation to time and frequency, were studied in patients with stable chronic heart failure. Compared with controls, circadian variations in autonomic parameters, either after training or after detraining, were maintained in chronic heart failure. Physical training produced significant increase in all time and frequency domain measures of heart rate variability predominantly assessing vagal activity and decrease in low/high-frequency ratio an index of sympatho-vagal balance, whilst the circadian variation of low- and high-frequency components was preserved following the same pattern the control group followed throughout the 24-hour period of recording.

These findings raise the possibility that the autonomic imbalance associated with chronic heart failure may in part be due to chronic physical deconditioning and may, at least partially, be reversible by exercise training programmes. These training-induced beneficial effects on adverse prognostic features (such as increased noradrenaline spillover and reduced heart rate variability) may lessen the predisposition to ventricular arrhythmias and reduce mortality in patients with chronic heart failure. The preservation of a circadian variation of low- and high-frequency components between patients after both training and detraining suggests that the syndrome of heart failure is characterised by a resseting of autonomic balance rather than autonomic neuropathy.

III. Animal studies have demonstrated that intermittent sympathomimetic stimulation with dobutamine produces benefits analogous to those of physical conditioning. An attempt was undertaken as closely as possible to imitate pharmacologically the stimulus to the β_1-

receptors of the cardiac myocyte produced by physical training. Short bursts of pharmacological ß-adrenergic stimulation with dobutamine were given in 10 patients with stable moderate to severe chronic heart failure with major end-points lymphocyte ß-receptor density, autonomic function and exercise tolerance. Results were compared with a control group of 10 patients where no dobutamine infusion was performed and major end-points reevaluated immediately and 6 weeks after the completion of pulsed inotrope therapy and in the control group of patients.

Intermittent, short duration pulsed inotrope therapy induced pharmacological conditioning with improved symptoms and exercise tolerance associated with improved autonomic balance (reduction of plasma noradrenaline concentrations and low/high-frequency ratio), ß-receptor up-regulation and enhanced chronotropic responsiveness (to either exercise or dobutamine) in patients with chronic heart failure. These beneficial effects persisted, to a great extent, for at least 6 weeks after pulsed inotrope therapy had been completed, thus encouraging us to reconsider the role of ß-receptor stimulants in patients with severe chronic heart failure by changing the method of administration.

IV. A more detailed understanding of the integrated pathophysiology undrelying both the progression of heart failure and the complex interaction between exercise responses and symptoms was attempted by studying the evolution of haemodynamic, neurohormonal and skeletal muscle abnormalities after myocardial infarction. The time course of skeletal muscle metabolic changes, autonomic function and central haemodynamics following extensive anterior myocardial infarction was described in 10 patients, using ^{31}P magnetic resonance spectroscopy to study forearm metabolism, heart rate variability and radiolabeled noradrenaline kinetics to assess sympatho-vagal balance, and pulsed-wave Doppler to estimate cardiac output. Results were compared with 22 normal subjects and 22 patients with stable chronic heart failure. Studies were performed 'early' (1-3 weeks), 'mid' (6-8 weeks) and 'late' (6-9 months) following a first extensive anterior myocardial infarction.

Four patients developed overt heart failure after the second study, while the remainder showed only asymptomatic left ventricular dysfunction. Skeletal muscle metabolism became abnormal late after the extensive myocardial infarction and metabolic abnormalities appeared to have no relationship with the development of the overt syndrome of heart failure. Thus, the maximal rate of oxidative ATP synthesis (reflecting mitochondrial oxidative capacity), calculated from post-exercise phosphocreatine kinetics, was lower than control in late post-myocardial infarction and established chronic heart failure groups. The initial rate of ATP turnover (which is inversely related to 'effective muscle mass'), calculated from initial exercise changes in pH and phosphocreatine, was significantly higher only in the early study, although the values for all post-myocardial infarction groups were numerically similar to the established chronic heart failure. On the contrary, patients who subsequently developed chronic heart failure showed persistent neurohormonal hyperactivation (expressed either with increased noradrenaline spillover or with predominance of the heart rate variability-derived indexes describing sympathetic drive) throughout the post-myocardial infarction period, even when asymptomatic, similar to that of patients with

established chronic heart failure. Similarly, a significantly lower response of cardiac output to exercise was detected in patients who subsequently manifested heart failure, even when examined during the asymptomatic stage of left ventricular dysfunction.

Skeletal muscle metabolism and autonomic function became abnormal after an extensive myocardial infarction. Skeletal muscle metabolic abnormalities were slow to develop and unrelated to the degree of failure, whereas early excessive neurohormonal activation seem to characterise patients who subsequently develop chronic heart failure. In addition, myocardial contractile reserve, evaluated during the asymptomatic period after an extensive myocardial infarction, was impaired in the subgroup of patients who manifested symptoms of chronic heart failure a few months later. These findings may allow more effective therapeutic strategies to be designed by looking beyond the conventional haemodynamic remedies for heart failure and by intervening earlier in the pathophysiological chain of events following an extensive myocardial infarction with therapeutic modalities (such as physical training) that could delay or even reverse the progress towards the clinical expression of chronic heart failure.

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