

**Exercise tolerance and skeletal muscle structure and function in patients with severe
chronic heart failure**

Submitted for the degree of Masters (Med) Exercise Science

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patients with severe chronic heart failure**

Kirsten Louise Derman

Dedication

To my loving husband Wayne,
and to Raine, our beautiful daughter,
thank you for your perfect love.

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Declaration

I, Kirsten Louise Derman, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work or any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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ABSTRACT

Fatigue and exercise intolerance are common symptoms experienced by patients with chronic heart failure (CHF). Historically it has been argued that central cardiopulmonary factors including pulmonary congestion and reduced lung compliance cause dyspnoea that limits the exercise tolerance of such patients. But recent studies have indicated that exercise capacity in patients with CHF may not be limited solely by central cardiorespiratory factors. Rather the focus has shifted to aspects of the peripheral circulation and skeletal muscle function as possible factors limiting the exercise tolerance of patients with CHF. However there are few studies describing both the structural and functional abnormalities in the skeletal muscle of patients with CHF.

In the first study of this dissertation, 11 patients with end-stage heart failure (NYHA class III-IV) and 10 healthy control subjects (C) underwent i) graded exercise to exhaustion for determination of peak oxygen consumption (VO_2 peak) and peak work load (WLpeak); ii) isometric and isokinetic tests of skeletal muscle function and iii) radionuclide angiography for determination of ejection fraction (EF%).

VO_2 peak (12.5 ± 1.0 vs 34.3 ± 3.5 mlO₂/kg/min; $p < 0.001$), WLpeak (73 ± 10 vs 224 ± 14 W; $p < 0.001$), total work performed by the quadriceps muscles (TWQ) in a 30 sec isokinetic test (TWQ; 1565 ± 166 vs 2892 ± 345 J; $p < 0.05$), and hamstring muscles (TWH) (TWH; 604 ± 163 vs 2003 ± 326 J; $p < 0.05$), maximum voluntary isometric contraction (MVC) of the quadriceps muscles (MVC; 134 ± 12 vs 194 ± 11 Nm; $p < 0.001$) and isokinetic peak torque of the quadriceps (PKTQ) (PKTQ; 133 ± 15 vs 203 ± 23 Nm; $p < 0.05$) and hamstring muscles (PKTH) (PKTH; 60 ± 8 vs 108 ± 16 Nm; $p < 0.05$) and time to fatigue during a test of isometric endurance (68 ± 12 vs 100 ± 10 sec; $p < 0.05$) were all significantly lower in patients with CHF.

However when corrected for the reduced lean thigh volume (muscle mass) in patients with CHF, PKTQ, PKTH and MVC were no longer different from control values. But the total work performed by the quadriceps and hamstring muscles in a 30 second isokinetic test was reduced even when corrected for the reduced lean thigh volume in patients with CHF. Furthermore, patients with CHF terminated progressive cycle exercise to exhaustion at heart rates, rates of ventilation, respiratory exchange ratios and blood lactate concentrations that were significantly lower than values achieved by control subjects during maximal dynamic exercise. These data suggest that skeletal muscle functional abnormalities including a decreased resistance to the development of fatigue exist in patients with severe CHF.

In the second study of this dissertation, 10 patients with CHF who participated in the first study and eight control subjects underwent a skeletal muscle biopsy of the vastus lateralis muscle for light and electron microscopic analysis.

Significant histological and ultrastructural changes were found in all SM biopsies from patients with CHF. These included atrophy and hypertrophy of fibres, fibre splitting, internalized nuclei, nuclear knots, moth-eaten fibres, increased lipid droplets. Electron microscopy showed a large variety of nonspecific abnormalities, including mitochondrial changes, Z-band degeneration and accumulation of intracellular glycogen. Ultrastructural morphometry revealed capillary basement membrane width significantly increased in the SM of patients with CHF, (409 ± 13 vs 121 ± 3 nm; $p < 0.01$). A novel, blinded, impartially scored method for grading SM pathology showed that SM biopsies of patients with CHF had higher scores for myopathic changes compared to C (12.0 ± 1.5 vs 1.6 ± 1.0 arbitrary units; $p < 0.05$). SM pathology score correlated significantly with VO_2 peak, WLpeak, and TWQ ($p < 0.05$ to $p < 0.02$) but not with EF%. EF% did not correlate with either VO_2 peak, WLpeak or TWQ.

These data support the hypothesis that: i) severe SM structural and functional abnormalities may limit exercise capacity in patients with CHF; ii) the severity of SM pathology but not resting systolic cardiac function, predicts exercise performance in patients with CHF.

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CHAPTER 1

LITERATURE REVIEW

EXERCISE INTOLERANCE IN PATIENTS WITH CHRONIC HEART FAILURE

THE PHYSIOLOGICAL RESPONSE TO EXERCISE IN PATIENTS WITH HEART FAILURE

I. Historical perspective of exercise intolerance in heart failure

Braunwald and Grossman (1992) defined heart failure as "the condition in which abnormality of myocardial function is responsible for the ventricles' inability to deliver adequate quantities of blood to the metabolizing tissues at rest or during normal activity". The most common cause of heart failure is loss of cardiac muscle secondary to ischaemia on the basis of coronary artery disease. Viral and other cardiomyopathies are more common in younger patients. Heart failure can also occur in persons with poorly controlled blood pressure or with valvular heart disease.

Of the many symptoms experienced by patients with heart failure, dyspnoea, fatigue and exercise intolerance are amongst the most common. Indeed, the first indication of heart failure is a diminished tolerance to physical exercise (Lewis 1933). Of such importance are symptoms of dyspnoea that functional capacity in patients with chronic heart failure was, and still is, measured in terms of the four-tiered classification of breathlessness (The Criteria Committee of the New York Heart Association Inc. 1964), which is subjective and only semi-quantitative. Only recently has exercise testing become more popular for the determination of functional capacity in patients with heart failure (Weber and Janicki 1985a; Lipkin et al. 1985).

Historically it was believed that central cardiorespiratory factors determined submaximal and maximal exercise capacity in patients with chronic heart failure (Weiss and Ellis 1935, Donald et al. 1961, Patterson et al. 1972, Bruce 1977, Clausen 1976, Reddy et al. 1988). As these patients had reduced cardiac output at rest and during exercise, it was thought that the heart was unable to deliver sufficient oxygen to the active skeletal muscles so that an oxygen deficiency in the active muscles caused fatigue. The focus of studies to determine mechanisms of fatigue at rest and during exercise in patients with heart failure was on pulmonary congestion, reduced lung compliance and dyspnoea. Even more recently, the majority of studies have continued to focus on the role of the heart and lungs in the limitation of exercise performance in patients with heart failure (Katz et al. 1989, Cowley et al. 1991, Moore et al. 1992, Myers et al. 1992).

However, since 1960 there has been evidence that signs and symptoms of heart failure could be absent even in the presence of severe heart disease (Eichna 1960). This led researchers to believe that central cardiovascular function may not necessarily be related to exercise capacity (Muller et al. 1992; Franciosa et al. 1981; Benje et al. 1980) and that chronic heart failure encompasses impairment of multiple organ systems (Rubin and Swan 1986) including skeletal

muscle (Lipkin et al. 1988; Minotti et al. 1991; Buller et al. 1991; Magnusson et al. 1994). Thus, a reasonable clinical definition of heart failure would be: a disorder of the heart resulting in impaired cardiac performance leading to reduced exercise tolerance particularly on exertion, associated with the activation of compensatory physiological reflexes leading to salt and water retention and further impairment of cardiac performance (Packer 1992).

II. Physiological factors contributing to exercise intolerance in patients with chronic heart failure

Although exercise intolerance is the most frequent symptom in patients with heart failure, the exact cause of exercise intolerance has yet to be identified. Whilst heart failure is a complex and multi-systemic disorder, and whilst the author appreciates that central and peripheral physiological systems cannot be entirely separated, the limitation of exercise performance in heart failure will be discussed under central (cardiorespiratory) factors and peripheral (blood flow and skeletal muscular) abnormalities which could possibly alter exercise tolerance in patients with chronic heart failure

1. Contribution of central cardiorespiratory abnormalities to exercise intolerance in patients with heart failure

(i) Left Ventricular Systolic Function

Left ventricular systolic function is compromised in chronic heart failure. It was thought that this would alter exercise tolerance in patients with heart failure. However, the poor relationship between maximal exercise capacity and indices of left ventricular (LV) function in patients with heart failure is now an established finding (Port et al. 1981; Baker et al. 1984; Franciosa et al. 1981; Meiler 1987; Higginbotham et al. 1983; Cowley et al. 1991; Benje et al. 1980; Myers et al. 1992; Reading et al. 1993). There is clearly no correlation between exercise capacity measured on a cycle ergometer or treadmill, and LV ejection fraction, nor any correlation between the degree of ventricular dysfunction and the clinical severity of chronic heart failure (Franciosa et al. 1979, 1981). A study by Benje et al. (1980) showed that 50% of patients with an ejection fraction of less than 30% had normal exercise capacity. Reading et al. (1993) also reported that there was no relationship between resting ejection fraction and peak aerobic power or resistance in the calf vasculature.

Furthermore, it was reported that patients with severe LV dysfunction increased their peak exercise performance after exercise training, whilst they did not show any change in LV ejection fraction or any reduction in wall motion abnormalities (Conn et al. 1982; Kellerman et al. 1986; Hoffman et al. 1987). These findings suggest that factors other than those relating to central cardiovascular function may have determined the beneficial adaptations to training. Regardless of these findings, the ejection fraction continues to be the most common measure of central cardiac performance and predictor of future survival in these patients (Higginbotham et al. 1983; Cohn et al. 1987; Szlachic et al. 1985; Swan 1986).

(ii) Cardiac Output

Cardiac output is reduced at rest and during exercise in patients with chronic heart failure. Furthermore, a lower proportion of total cardiac output is delivered to skeletal muscle during exercise in patients with heart failure (Zelis and Flaim 1982). Bain et al. (1990) demonstrated that exercise time to exhaustion measured during incremental exercise in patients with heart failure, cannot be predicted from resting cardiac output. However, maximal cardiac output and the ability to increase cardiac output during exercise, do correlate with exercise capacity. Muller et al. (1992) showed that blood flow to the gut, kidneys, and limbs was reduced in patients with chronic heart failure. The lack of any correlation between limb blood flow and cardiac output helps to explain the lack of correlation between cardiac output and symptoms during exercise.

Further evidence that cardiovascular function does not determine exercise capacity in patients with heart failure is the observation that improvement of left ventricular function by acute ingestion of inotropic drugs (Maskin et al. 1983), vasodilators (Wilson et al. 1984b), or repair of valvular stenosis (Marzo et al. 1991) does not result in an immediate normalization of exercise capacity or of their ventilatory response during exercise, despite marked improvements in cardiac output (Marzo et al. 1992; Drexler et al. 1991a).

(iii) Blood Pressure

Regulation of blood pressure and temperature are major cardiovascular challenges which arise during muscular work and which may conflict with the need to perfuse the active muscles. Normal persons actively regulate mean arterial blood pressure during exercise by increasing skeletal muscle vascular tone when cardiac output decreases (Strandell and Shepherd 1967; Mack et al. 1988). In patients with heart failure, this reflexly-mediated peripheral vasoconstriction is thought to maintain blood pressure during dynamic exercise when cardiac output has reached its maximum

value (Sullivan et al. 1989). As a result, blood flow to the active muscles is restricted in order to maintain blood pressure when cardiac output is limited.

Heart rate and mean systemic arterial blood pressure increase less during dynamic exercise in patients with heart failure compared to control subjects and the increase in stroke volume in the patients is also minimal (Wilson et al. 1984a). Despite this abnormal response, mean systemic blood pressure does not relate directly to exercise capacity (Higginbotham et al. 1983; Bain et al. 1990), suggesting that exercise intolerance in these patients is influenced by factors other than peak systolic blood pressure (Sullivan et al. 1988b; Franciosa et al. 1984).

(iv) Pulmonary Haemodynamics

It was originally thought that increased pulmonary capillary wedge pressure during exercise caused by left ventricular failure caused dyspnoea, which in turn limited exercise performance (Weber et al. 1982). However, the elevated pulmonary capillary wedge pressures at rest in patients with heart failure increase to the same levels during submaximal and maximal exercise (Szlachcic et al. 1985), and these elevated levels appear to be well-tolerated (Roubin et al. 1990). Massie (1988) found pulmonary capillary pressures to be no higher in patients with heart failure whose exercise tolerance was limited by dyspnoea compared to patients limited by leg fatigue. These findings indicate that mechanisms other than pulmonary capillary wedge pressure appear to be important in limiting exercise performance in patients with chronic heart failure (Sullivan et al. 1988b; Franciosa et al. 1984).

(v) Pulmonary Ventilation

Patients with chronic heart failure have altered ventilatory responses to graded exercise. Hyperventilation is commonly observed during exercise in patients with heart failure and has been attributed to decreased lung compliance and regional ventilation-perfusion mismatch in the lungs as a result of the reduced cardiac output (Wada et al. 1992; Myers et al. 1991) and increased dead space volume (Weber et al. 1982; Sullivan et al. 1988b). Despite this abnormal ventilatory response to exercise and decreased maximal ventilation (Rubin and Swan 1986), the normal control of ventilation by VCO_2 is present in patients with chronic heart failure (Sullivan et al. 1988b; Fink et al. 1986; Myers et al. 1992).

Although it is unclear to what extent these ventilatory abnormalities influence exercise performance in patients with chronic heart failure (Myers et al. 1992), it has been suggested that

leg fatigue causes termination of exercise before there is a substantial encroachment on ventilatory reserve in patients with severe heart failure (Rubin and Swan 1986; Myers et al. 1992).

It is possible that abnormal respiratory muscle function may contribute to breathlessness and ventilatory abnormalities in these patients (Zelis et al. 1988). Nishimura et al. (1994) reported that inspiratory muscle strength was impaired in patients with severe congestive heart failure and may limit exercise in these patients. Furthermore, 'periodic breathing' has been described in patients with congestive heart failure at rest and during exercise (Kremser et al. 1987; Ribiero et al. 1987). This has been attributed to unstable ventilatory control caused by poor circulation and to delayed transmission of humoral ventilatory stimuli to the chemoreceptors (Lange and Hecht 1962; Cherniack and Longobardo 1973). However, the exact mechanism for the periodic breathing in patients with heart failure is still unknown, although a central (cardiovascular) mechanism is still favoured (Ben Dov et al. 1992). Little is known about the time course, exact changes, causative factors, or the mechanisms of these ventilatory alterations. It is not known whether these alterations in pulmonary ventilation are the cause or effect of the exercise intolerance.

(vi) Peak Oxygen Consumption (VO_2 peak)

Reduced VO_2 peak in patients with heart failure has been repeatedly confirmed (Franciosa et al. 1979; Higginbotham et al. 1983; Weber et al. 1982; Myers et al. 1992; Miyagi et al. 1993). However, the exact cause of this finding has not been identified. Decreased oxygen consumption in patients with chronic heart failure implies that either O_2 supply or O_2 utilization by the active skeletal muscle is altered. Alternatively, the muscle's capacity to exercise could be impaired even in the face of an adequate oxygen supply.

Wade and Bishop (1962) suggested that the primary limitation of VO_2 peak in patients with heart failure is a relatively reduced pulmonary blood flow. However, many investigators have since shown that arterial oxygen tension and systemic arterial O_2 saturation remains normal even at peak exercise in patients with chronic heart failure (Franciosa et al. 1984; Roubin et al. 1990; Sullivan et al. 1988b). This suggests that pulmonary function does not limit VO_2 peak in these patients, and that peripheral alterations may indeed influence peak oxygen consumption in these patients. These theories are supported by the following important findings:

(a) Weber et al. (1982) demonstrated that although peak cardiac output and peak VO_2 are linked, as the severity of heart failure worsens, the slope of the cardiac output response with increasing VO_2 decreases. This indicates that the normal relationship between cardiac output and oxygen

consumption is altered in these patients, possibly due to increased oxygen extraction at the periphery from a reduced (muscle) blood flow.

(b) Thompson et al. (1974) and Sullivan et al. (1990) reported that femoral venous PO₂ does not decrease below the critical level of 10mmHg during exercise in both normal subjects and in patients with chronic heart failure. These findings suggest that muscle fatigue may not be caused by an inadequate oxygen supply to the active muscle.

(c) Failure to observe an improvement in oxygen uptake by skeletal muscle during exercise even when oxygen availability is enhanced by pharmacological intervention in patients with chronic heart failure (Drexler et al. 1987a,b) indicates a change in the properties of skeletal muscle itself in these patients.

(d) Moore et al. (1992) reported that maximal exercise performance on a cycle ergometer was acutely enhanced compared to exercise with room air, when patients with heart failure inspired oxygen at a concentration of 50% O₂. This was explained by an increased efficiency during exercise, so that there was a decreased ventilatory and circulatory demand at a given workload, while O₂ delivery to muscle was maintained (Moore et al. 1992). However, this does not prove that oxygen supply to the active muscle was limiting during exercise.

(e) Jondeau et al. (1992) measured VO₂ peak during maximal exercise with lower limbs and with lower limbs plus upper limbs in patients with heart failure. Patients were able to increase VO₂ peak when exercising with both lower and upper limbs. This finding was not observed in the control group of healthy subjects. This indicates that the exercise intolerance in cardiac patients during lower limb exercise was not due to a central limitation, as VO₂ peak increased when additional skeletal muscle was recruited in these patients.

(f) Recent reports indicate that VO₂ peak is predicted by muscle strength in patients with chronic disease. Isometric maximal voluntary contraction (MVC) of the quadriceps muscle correlates to peak VO₂ during exercise in patients with chronic heart failure (Lipkin et al. 1988). This finding indicates that skeletal muscle function can predict maximal exercise performance. Similarly, isokinetic quadriceps muscle strength correlates to peak VO₂ in patients with renal failure (Diesel et al. 1990). Therefore peak VO₂ is probably indicative of peripheral, skeletal muscular rather than central cardiovascular function in this group of patients (Noakes 1988; Kempeneers et al. 1989; Diesel et al. 1990).

In light of the above-mentioned findings, the search for mechanisms of exercise intolerance in patients with heart failure has shifted from the central cardiovascular system to the peripheral musculoskeletal system.

2. Contribution of peripheral abnormalities to exercise intolerance in patients with heart failure

(1) Physiological alterations in the periphery during exercise in patients with chronic heart failure are:

(i) Alteration of blood flow to skeletal muscle

Peripheral blood flow is lower during progressive dynamic and during sustained isometric exercise in patients with chronic heart failure compared to normal controls (Wilson et al. 1983, 1984a,b; Zelis et al. 1974; Longhurst et al. 1976). A lower proportion of total cardiac output is delivered to exercising skeletal muscle in patients with heart failure. This occurs as a compensatory mechanism to prevent hypoperfusion of important non-exercising areas (brain, kidney) or to preserve arterial blood pressure (Sullivan et al. 1989; Zelis and Flaim 1982) or as a result of increased skeletal muscle vascular resistance (Zelis and Flaim 1982). Recently Yamabe et al. (1992) reported that blood flow to skeletal muscle ($L/min/m^2$) during submaximal cycle exercise is higher in NYHA class III patients than to skeletal muscle of patients with less severe heart failure. These investigators suggest that the relatively increased skeletal muscle blood flow patients with severe heart failure plays a role to compensate for the insufficient cardiac output response in these patients.

Many researchers have reported that systemic vascular resistance is significantly higher during exercise in patients with heart failure compared to control subjects (Sullivan et al. 1989; Szlachcic et al. 1985; Franciosa et al. 1984). But is this increased vascular resistance responsible for exercise intolerance in patients with chronic heart failure? Rubin and Swan (1986) suggested that there is a normal decrease in the vascular resistance of the vascular beds in the exercising muscles in proportion to the intensity of exercise in patients with heart failure.

Impaired vasodilation during exercise is an important factor responsible for the reduced maximal limb blood flow in patients with heart failure (Sullivan et al. 1988b; Zelis et al. 1986; Wilson et al. 1986; Mancini et al. 1987). But the pathophysiological cause of decreased muscle blood flow during exercise has not been clearly defined. Possible factors limiting blood flow to the skeletal

muscle during exercise include alterations in vascular stiffness, in neurohumoral activation, in blood vessel vasodilator capacity and in flow-dependent dilation in patients with heart failure. These factors may play an important role in the natural history of the disease, but may not be critical to the impaired exercise capacity of these patients (Cohn 1992).

(a) Alterations in vascular stiffness

Zelis et al. (1968) studied the effects of vasodilator stimuli on peripheral resistance vessels in patients with congestive heart failure and found that approximately one-third of the reduced maximum vasodilator response during exercise can be attributed to an increased sodium content of the arterial wall. However, vascular resistance in the leg vessels remains increased even in patients receiving optimal treatment with diuretics (Le Jemtel 1986). This finding may indicate that other factors besides increased sodium content in the arterial wall are responsible for impaired vasodilation in patients with chronic heart failure.

(b) Neurohumoral activation

Plasma epinephrine concentrations are elevated at rest in patients with congestive heart failure (Francis et al. 1984; Riley et al. 1990) and during low intensity exercise (Francis et al. 1982), but are not significantly different from controls during moderate and strenuous exercise (Kirlin et al. 1986). Wilson et al. (1984b) demonstrated that at the same relative workload, plasma norepinephrine concentrations in patients with chronic heart failure were actually lower than in normal subjects, suggesting that the extent to which the sympathetic adrenergic system is activated during exercise may be substantially less in patients with heart failure than in normal subjects (Wilson et al. 1989). These findings indicate that factors other than altered plasma epinephrine concentrations might be responsible for the decreased blood flow to skeletal muscle in patients with heart failure.

Plasma renin concentrations are elevated at rest and during exercise in patients with heart failure (Cody et al. 1992) and represent compensatory activation of the renin-angiotensin-aldosterone system (Kirlin et al. 1986). Compared to control subjects, myocardial secretion of atrial natriuretic factor is increased 5- to 10- fold at rest (Bates et al. 1986; Leinonen et al. 1988; Cody et al. 1986), and during exercise in patients with heart failure (Cody et al. 1992). Atrial natriuretic factor can suppress the renin-aldosterone pathway (Cody et al. 1992:86) and during exercise may minimize the effects of angiotensin II mediated vasoconstriction (Cody et al. 1992).

(c) Blood vessel vasodilator capacity

Wilson et al. (1984b; 1985) examined the acute effects of the vasodilators dobutamine, dopamine, and phosphodiesterase on maximal exercise performance in patients with chronic heart failure and found no improvement in exercise performance even when blood flow to the legs was acutely increased by these agents. Nor did increased blood flow to exercising muscle induced by hydralazine appear to reduce femoral venous lactate concentrations, or the degree of acidosis or fatigue in patients with heart failure (Wilson et al. 1983). These results indicate that exercise performance was not dependent on muscle blood flow, as it failed to increase with an *acute* increase in muscle blood flow (Wilson et al. 1984a).

Drexler et al. (1991b; 1992) report that *chronic* administration of angiotensin converting enzyme (ACE) inhibitors can partially reverse mitochondrial abnormalities in patients who have depressed oxidative capacity of skeletal muscle during exercise. Chronic ACE inhibition not only increases skeletal muscle blood flow at maximal exercise (Mancini et al. 1987), but also improves functional capacity in these patients by acting primarily on the peripheral vasculature without changing intrinsic myocardial function (McGrath et al. 1985). These results suggest that there is a gradual reduction in structural vascular abnormalities by the chronic effect of ACE inhibition, and that this improves exercise performance.

It is important to note that some patients with chronic heart failure have normal skeletal muscle blood flow at rest and during effort, despite markedly impaired effort tolerance (Wiener et al. 1986; Massie et al. 1987a). Thus impaired exercise tolerance might be related to altered skeletal muscle metabolism, associated with reduced muscle mass and strength, rather than altered skeletal muscle perfusion, at least in these patients (Mancini et al. 1988; Massie et al. 1987a).

(d) Flow-dependent dilation

Recent studies by Drexler et al. (1989) and Anderson & Mark (1989) indicate that flow-dependent dilation occurs in humans both in peripheral and coronary arteries. Endothelin, an endothelium-derived peptide, is a circulating hormone with potent vasoconstrictor properties. Two- to threefold increases in circulating endothelin concentrations have been demonstrated in patients with chronic congestive heart failure, and concentrations increase progressively with the severity of congestive heart failure (Rodeheffer et al. 1992). Modulators such as endothelium-derived relaxing factor (EDRF) (Lerman et al. 1992), atrial natriuretic factor (ANF) (Margulies et al. 1991) and

endothelium-derived ANF-like peptide C-type natriuretic peptide (Stingo et al 1992) have been proposed as therapeutic regimens to counteract the vasoconstrictor effects of endothelin.

It has been suggested that chronic high blood flow states result in endothelial changes that lead to enhanced release of EDRF (Zelis et al. 1988). EDRF mediates vasodilation, a characteristic effect of physical conditioning. Chronic deconditioning, accompanied by lower peripheral flow, may reduce the endothelium-derived vasodilator capacity, eventually leading to a reduction in vessel diameter. Drexler and Lu (1992) found that reduced receptor-mediated endothelium-dependent dilation impairs exercise-induced vasodilation in skeletal muscle of rats. These results highlight the importance of the endothelium for the vascular response in both normals and in patients with heart failure. Further research on the importance of these variables in determining blood flow in patients with heart failure and their impact on exercise tolerance and conditioning is clearly important. However, the hypothesis that underperfusion of muscle contributes to exertional fatigue in heart failure remains to be fully established.

Reduced blood flow to skeletal muscle during maximal exercise appears to be confined to the more oxidative skeletal muscle fibres. In a rat model of chronic heart failure, blood flow to glycolytic (Type II) muscle fibres increased adequately with exercise while ability to increase blood flow to oxidative (Type I) fibres was impaired (Drexler et al. 1987a). This finding suggests that arterial blood flow to Type I fibres might be responsible for some of the morphological changes in these fibres in the skeletal muscle of patients with chronic heart failure, namely the Type II fibre predominance.

(ii) Reduced blood flow causing altered skeletal muscle metabolism

It has been suggested that reduced muscle blood flow at rest and during exercise in patients with heart failure (Sullivan et al. 1989; Zelis et al. 1975; Massie et al. 1987a,b, 1988; Wiener et al. 1986; Wilson et al. 1984b; Walker et al. 1982) might be important in determining the metabolic response to exercise, characterized by increased glycolytic metabolism and decreased oxidative phosphorylation in the skeletal muscles of patients with chronic heart failure. However, Weiner et al. (1986) reported that the reduced blood flow to exercising skeletal muscle was not a consistent finding in forearm muscles where metabolism was altered. Massie et al. (1988) established that metabolic abnormalities are unrelated to blood flow by comparing the effects ischaemic forearm exercise in patients with heart failure and in normal control subjects. While the brachial artery was temporarily occluded during submaximal finger flexion, patients utilized phosphocreatine (PCr) at a faster rate compared to controls despite performing much less work, and pH levels fell

substantially. As blood flow was not different, results suggest that a functional abnormality of the skeletal muscle exists in patients with congestive heart failure. Buller et al. (1991) measured fatigability of adductor pollicis muscle during supramaximal repetitive stimulation of the ulnar nerve during circulatory occlusion in patients with severe heart failure and in normal controls. The effect of ischaemia was greater in patients with severe (Class IV) heart failure than in patients with mild to moderate heart failure or in normals and the investigators attributed this to impaired skeletal muscle metabolism. Marie et al. (1990) confirmed this when they reported that the rate of PCr resynthesis in calf muscle of patients with congestive heart failure was similar following either aerobic or ischaemic exercise.

(iii) Oxygen extraction and utilization

Despite decreased blood flow during submaximal exercise, it has been demonstrated that the skeletal muscles of patients with chronic heart failure can compensate by increasing oxygen extraction, hence increasing the arterio-venous oxygen (A-VO₂) difference (Wilson et al. 1984a). However, Roubin et al. (1990) observed that although the A-VO₂ difference across the leg during exercise was greater in patients with heart failure than in normal persons, VO₂ peak was 40% lower, suggesting that the greater A-VO₂ difference does not compensate completely for the reduction in skeletal muscle blood flow. Alternatively skeletal muscle weakness may prevent patients with heart failure from reaching high levels of exercise at which higher VO₂ values would be measured. Wilson et al. (1984b) showed that maximal leg blood flow and maximal leg oxygen uptake in patients with chronic heart failure were most markedly reduced in patients with the poorest exercise tolerance. These findings suggest that either the skeletal muscle cannot fully utilize the delivered oxygen or that oxygen availability or diffusion to the skeletal muscles is impaired in these patients. However, these studies do not exclude the possibility that muscle weakness limits exercise tolerance before an oxygen limitation develops.

(iv) Blood lactate concentrations

It is usually argued that skeletal muscle hypoperfusion during exercise contributes to the early onset of skeletal muscle lactate production (Weber and Janicki 1985a,b; Wilson 1984b; Wiener et al. 1986; Adamopoulos & Coats 1991) and that increasing exercise intolerance is associated with progressively earlier increases in mixed venous blood lactate concentrations in patients with chronic heart failure (Wilson et al. 1984; Weber et al. 1982, Weber and Janicki 1985a). Furthermore, it has been demonstrated that venous blood lactate accumulation patterns correlate with severity of circulatory failure at rest (Meakins and Long 1927) and with severity of heart failure

during exercise (Weber and Janicki 1985a). Increased lactate concentration is thought to be a contributing factor to fatigue and dyspnoea experienced during exercise in patients with chronic heart failure (Hanson 1994).

(2) Skeletal muscle abnormalities

(1) Histological and biochemical abnormalities in skeletal muscle biopsies of patients with chronic heart failure

Recent studies report that patients with heart failure identify leg fatigue as the limiting factor during exercise (Roubin et al. 1990; Kitzman et al. 1991; Myers et al. 1992) and that the stride length of walking is reduced in patients with chronic heart failure (Davies et al. 1992). The study of skeletal muscle as the possible cause of exercise intolerance in these patients has gained popular interest.

A review of histological and biochemical findings of previous studies of skeletal muscle samples from patients with chronic heart failure is presented in *Table 1.1*. Abnormalities of skeletal muscle fibre morphometry, cellular organelles, capillary structure and biochemical alterations have been described in these patients.

(i) Fibre morphometry

Atrophy and hypertrophy of both Type I fibres (Lipkin et al. 1988; Sullivan et al. 1990; Poole-Wilson et al. 1988) and Type II fibres (Lipkin et al. 1988; Dunnigan et al. 1987; Poole-Wilson et al. 1988; Mancini et al. 1989; Wilson et al. 1992) have been reported in patients with CHF. The percentage of Type II fibres is increased in patients with CHF (Mancini et al. 1989; Sullivan et al. 1990; Drexler et al. 1992). In skeletal muscle biopsies from vastus lateralis in normal subjects, Type I muscle fibres constitute less than 55% of the fibres and Type II muscle fibres less than 80% (Dubowitz 1985). Fibre predominance occurs if these figures are exceeded.

Recent work by Lipkin et al. (1988), Mancini et al. (1991a) and Minotti et al. (1992) suggests that skeletal muscle atrophy occurs early in the course of congestive heart failure and might play a role in the reduction of functional capacity in patients with chronic heart failure. It is thought that compared to normal muscle, atrophied muscle is subjected to a greater workload per remaining fiber when faced with a given external load and therefore develops greater reductions in PCr and intracellular pH (Mancini et al. 1992). Mancini et al. (1992) recently tested this hypothesis by

Table 1. Review of histological and biochemical findings in studies of skeletal muscle biopsies in patients with chronic heart failure.

| Structure | Finding | Study |
|--|------------------------|--|
| <u>Fibre Morphometry</u> | | |
| Type I CSA | increased | Sullivan et al. 1990. |
| | decreased | Lipkin et al. 1988; Poole-Wilson et al. 1988. |
| Type II CSA | decreased | Dunnigan et al. 1987; Lipkin et al. 1988; Wilson et al. 1992; Poole-Wilson et al. 1988. |
| Type I % | increased decreased | Mancini et al. 1989. Mancini et al. 1989; Sullivan et al. 1990; Drexler et al. 1992. |
| Type II % | increased | Mancini et al. 1989; Sullivan et al. 1990. |
| <u>Cellular organelles</u> | | |
| mitochondrial volume density | decreased | Drexler et al. 1992. |
| surface area of mitochondrial cristae | decreased | Drexler et al. 1992. |
| Subsarcolemmal mitochondrial aggregates | increased | Dunnigan et al. 1987; Smith et al. 1976. |
| interstitial cellularity | increased | Lipkin et al. 1988. |
| <u>Capillaries</u> | | |
| capillary density | normal | Lipkin et al. 1988; Mancini et al. 1989. |
| capillary density | decreased | Sullivan et al. 1990; Drexler et al. 1992. |
| basement membranes | thickened | Longhurst et al. 1975. |
| <u>Intracellular lipid content</u> | | |
| | normal | Drexler et al. 1992. |
| | increased | Dunnigan et al. 1987; Lipkin et al. 1988, Smith et al. 1976. |
| <u>Intracellular glycogen content</u> | | |
| | normal | Drexler et al. 1992. |
| | decreased | Sullivan et al. 1990. |
| <u>Glycolytic enzyme activity</u> | | |
| | normal | Mancini et al. 1989; Sullivan et al. 1990. |
| <u>Oxidative enzyme activity</u> | | |
| cytochrome oxidase | decreased | Drexler et al. 1992; Ralston et al. 1991. |
| | normal | Lipkin et al. 1988. |
| citrate synthase | decreased | Mancini et al. 1989; Sullivan et al. 1990; Ralston et al. 1991; Wilson et al. 1992. |
| succinate dehydrogenase | decreased | Mancini et al. 1989; Sullivan et al. 1990; Ralston et al. 1991. |
| β -hydroxyacyl CoA dehydrogenase | decreased | Mancini et al. 1989; Sullivan et al. 1990. |
| succinate cytochrome reductase | normal | Lipkin et al. 1988. |

Abbreviations: CSA, cross sectional area.

correlating calf muscle volume with the metabolic response of the calf muscle to exercise during supine plantar flexion against increasing force. They discovered that skeletal muscle atrophy contributed significantly to the abnormal metabolic response to exercise, but that the correlation was relatively weak, suggesting that increases in muscle mass would produce only modest improvements in exercise capacity, and that the primary factor causing reduced pH and PCr during exercise is not skeletal muscle atrophy.

A possible cause of skeletal muscle atrophy in patients with chronic heart failure is chronic malnutrition and deconditioning due to reduced levels of habitual activity. Carr et al. (1989) reported that 50% of 48 patients in severe heart failure were malnourished, defined by a decrease in percent body fat, a decreased weight to height index, or reduced serum albumin concentrations. However, they did not measure skeletal muscle volume. However, Mancini et al. (1992) showed that muscle atrophy was not usually associated with signs of severe malnutrition and that protein synthetic function was maintained in heart failure. They also reported that anorexia was not the sole aetiological factor for the muscle atrophy; other factors include inactivity, an increased catabolic state due to heightened sympathetic stimulation and increased serum concentrations of cortisol, ACTH, and tumor necrosis factor (Peterson et al. 1988; Levine et al. 1990; Francis et al. 1990).

Diaphragmatic muscle atrophy has been described in patients who are chronically ill and in patients with chronic obstructive lung disease who have lost weight (Arora and Rochester 1982; Thurlbeck 1978). Indeed, intrinsic respiratory muscle changes may occur in patients with heart failure and could contribute to the generation of dyspnoea during exercise, and indeed to Cheyne-Stokes breathing.

Gibson et al. (1987) reported that immobility in patients with heart failure is associated with a 25% fall in protein synthesis in quadriceps muscle. However, as skeletal muscle regains normal protein synthesis after heart transplantation (Morrison et al. 1988), even though patients remain relatively immobile after heart transplantation, immobility cannot be the sole cause of this skeletal muscle abnormality.

Although atrophy may be caused by inactivity and bedrest, Poole-Wilson et al. (1988) reported that histological abnormalities seen in patients with chronic heart failure are not typical of the changes

seen as a result of either prolonged bedrest or of reversible ischaemia caused by peripheral vascular disease.

(ii) Cellular organelles

Drexler et al. (1988) reported reduced number and size of mitochondria, as well as a diminished number of cristae in the mitochondria of patients with heart failure, a finding consistent with diminished oxidative enzyme capacity.

Increased subsarcolemmal mitochondrial aggregates have been reported in children with cardiomyopathy and chronic heart failure (Dunnigan et al. 1987). Conversely, cardiac conduction defects have been described in many patients with mitochondrial myopathy (Karpati et al. 1973; Olson et al. 1972), suggesting that the myopathy is generalized (Bussieres et al. 1993).

(iii) Capillary abnormalities

Longhurst et al. (1975) studied biopsies from pronator teres muscle and found capillary basement membrane thickening in patients with heart failure compared to a control group. Wroblewski et al. (1992) documented increased capillary basement membrane thickening in the skin in patients with chronic heart failure and suggested that increased venous pressure due to heart failure and abnormal baroreceptor-mediated arteriolar vasodilation (Kassis 1989) raised capillary pressures in these patients. This enhanced stress could eventually lead to increased thickness of these basement membranes. Some studies suggest that basement membrane thickening is a result of repeated episodes of cell death and cell regeneration and may decrease diffusion across the capillary (Toussaint and Dustin 1963; Vracko 1970). However, Alpert et al. (1972) studied increased basement membrane thickness in diabetes and suggested that the increased thickness facilitates rather than retards oxygen diffusion.

(iv) Intracellular lipid and glycogen content

Intracellular lipid content has been reported to be normal (Drexler et al. 1992) or increased (Dunnigan et al. 1987, Lipkin et al. 1988). Increased intracellular lipid is a common non-specific finding in many muscle disorders. Intracellular glycogen concentrations may be normal (Drexler et al. 1992) or decreased (Sullivan et al. 1990).

(v) Glycolytic and mitochondrial enzyme activities

Whilst the skeletal muscle glycolytic enzyme activities are not reduced in CHF (Mancini et al. 1989; Sullivan et al. 1990), reduced activities of the enzymes involved in aerobic metabolism (succinate dehydrogenase and cytochrome oxidase) is a common finding in these patients (Drexler et al. 1991; Massie et al. 1987a,b; Mancini et al. 1988; Ralston et al. 1991; Wiener et al. 1986). Some researchers have found respiratory exchange ratios to be lower at maximal and at submaximal exercise in patients with chronic heart failure compared to controls (Myers et al. 1992; Riley et al. 1990). The decreased values in the patients could be explained by an increased reliance on fat, as opposed to carbohydrate metabolism in these patients in an effort to preserve skeletal muscle glycogen stores (Costill et al. 1977). Increased fat utilization is thought to be related to elevated noradrenaline concentrations at rest (Francis et al. 1982, 1984; Riley et al. 1990) causing elevated serum free fatty acid and glycerol concentrations (Riley et al. 1990).

An earlier fall in intracellular pH in response to submaximal and maximal exercise suggests accelerated rates of glycogenolysis and glycolysis (Sullivan et al. 1988c; Musch et al. 1986), thereby contributing to a reduced efficiency of contraction in the skeletal muscle of these patients leading to early fatigue (Bexton et al. 1983; Norgaard et al. 1990; Poole-Wilson et al. 1988; Drexler et al. 1992). Furthermore, Norgaard et al. (1990) reported that reduced Na/K pump activity in skeletal muscle in patients with chronic heart failure leads to a decreased sodium gradient across the cell membrane, causing intracellular accumulation of calcium and hydrogen. The compromised function of the mitochondria by altered handling of calcium ions within the cell may result in increased levels of ADP and P_i at any given workload during exercise (Dawson et al. 1978; Karlsson and Saltin 1970; Harmansen 1981). Thus, the onset of fatigue in these patients could be a consequence of ATP supply not being able to meet demand in the active muscles during exercise. Qualitative changes in skeletal muscle, such as an altered myosin isoform expression, may also change the skeletal muscle contractile function in these patients (Sullivan et al. 1990b).

(vi) Relation of abnormal skeletal muscle histology to the impaired exercise tolerance in CHF.

The studies reviewed above have documented changes in the skeletal muscles in CHF mainly on light microscopic analysis. Few studies have yet provided a detailed description of the ultrastructural findings. Although Drexler et al. (1992) reported a relationship between mitochondrial volume density and peak oxygen consumption in patients with CHF, the relationship

of skeletal muscle histological abnormalities to the impaired exercise tolerance of these patients has not been more fully defined.

(vii) Phosphocreatine recovery time

Mancini et al. (1992) reported that PCr recovery time after exercise, rather than muscle volume, correlated with the P_i-to-PCr and pH response during exercise in these patients, emphasizing that abnormal muscle metabolism, rather than atrophy per se, is probably the dominant mechanism for reduced exercise tolerance in patients with heart failure.

(2) Oxidative enzyme activity

Reduced oxidative capacity of skeletal muscle enzymes involved in aerobic metabolism, including succinate dehydrogenase and cytochrome oxidase, is a common finding in patients with heart failure (Drexler et al. 1991; Wilson et al. 1983, 1984; Massie et al. 1987a,b; Mancini et al. 1988; Ralston et al. 1991; Yancy et al. 1989; Wiener et al. 1986). Drexler et al. (1988) reported a close relationship between the oxidative capacity of skeletal muscle and VO₂ peak during exercise in patients with chronic heart failure, and suggested that the extent of alteration of skeletal muscle metabolism was related to exercise capacity in these patients. Furthermore, Stratton et al. (1994) reported that training improved oxidative capacity of forearm skeletal muscle, indicating that the impaired oxidative capacity may be due to inactivity and responds to a period of training. However, Lipkin et al. (1988) reported that skeletal muscle oxidative enzyme activity was within the normal range in patients with severe chronic heart failure. This finding is supported by Minotti et al. (1991b; 1993), who reported that since oxygen extraction by skeletal muscle is near complete in these patients, skeletal muscle metabolic disorders are not likely to substantially limit maximal exercise performance.

(3) Increased blood concentrations of tumor necrosis factor

Increased circulating concentrations of tumor necrosis factor (TNF) in skeletal muscle of patients with heart failure have been measured. However, this cytokine is not present in all patients with cardiac abnormalities (McMurray et al. 1991; Levine et al. 1990). Furthermore, Drexler et al. (1991b) recently reported that circulating concentrations of tumor necrosis factor do not correlate with the extent of skeletal muscle damage.

In summary, few studies have studied skeletal muscle abnormalities and whilst some studies have documented gross ultrastructural changes in the skeletal muscles of patients with chronic heart failure (Lipkin et al. 1988; Drexler et al. 1992), few studies have yet provided a detailed description of these changes or have provided adequate control groups to establish the specificity of these changes to heart failure.

III. Skeletal muscle function in patients with chronic heart failure

(1) Isometric skeletal muscle function

Several studies have reported that skeletal muscle function is impaired in patients with heart failure, even when the heart failure is mild (Lipkin et al. 1988; Minotti et al. 1991a; Buller et al. 1991). Lipkin et al. (1988) documented that isometric maximal voluntary contraction of quadriceps muscles of heart failure patients was 55% of the predicted strength for an age-, mass- and sex-matched population even after taking into account the decrease in cross-sectional area of the muscle, but no control group was actually tested.

Muscle cross-sectional area in healthy subjects is directly related to force development (Schantz et al. 1983; Maughan 1984) and it has been assumed that decreased cross-sectional area of skeletal muscle causes decreased skeletal muscle strength in patients with heart failure (Magnusson et al. 1994).

Buller et al. (1991) reported that supramaximal repetitive stimulation of a large muscle mass (quadriceps) caused rapid onset of fatigue and reduced isometric force production in patients with severe heart failure compared to patients with mild heart failure and controls, whereas force production and fatigue of adductor pollicis was not different between groups. However, the maximal isometric force production per unit muscle cross-sectional area was within the normal range (according to norms of Chapman et al. 1984). This led to the conclusion that the reduction in force for the larger muscle mass was not due to impaired force production by the myofibril. Minotti et al. (1991b) reported that maximal voluntary contraction was not different in patients compared to control subjects even though maximal cross-sectional area of the knee extensors was significantly smaller in the patients. They also found a strong correlation between isometric strength and maximal cross-sectional area of the thigh muscles. In addition, Magnusson et al. (1994) found that although the isometric strength of the quadriceps femoris muscle of these patients was 14% less than age-matched controls, the tension per unit of muscle cross-sectional area was similar between groups, which is in accordance with the report of Buller et al. (1991).

(2) Isokinetic skeletal muscle function

Minotti et al. (1991b) measured peak torque and endurance during isokinetic knee extensions in these patients and found that dynamic muscle endurance was significantly lower in patients compared to controls. Furthermore there was only a weak correlation between dynamic endurance and knee extensor cross-sectional area in these patients (Minotti et al. 1993). Also, Magnusson et al. (1994) reported a markedly lower dynamic endurance capacity of the quadriceps femoris muscles in patients with chronic congestive heart failure compared to age-matched controls, but there was no difference in peak tension per unit of cross-sectional area of the skeletal muscle. Thus it appears that diminished skeletal muscle endurance capacity during repetitive exercise is the most consistent finding in these patients, and that a reduction in muscle strength is not always a consistent finding, especially when the cross sectional muscle area is taken into account (Minotti et al. 1991b, 1993; Buller et al. 1991).

(3) Skeletal muscle recruitment

It has been suggested that central nervous system inhibition of skeletal muscle afferents may limit exercise during hypoxia or hypoperfusion in normal subjects (Hochachka and Dunn 1983; Green 1990; Garner et al. 1990). It is not known whether this is due to a feedforward or feedback mechanism from damaged or hypoxic skeletal muscle, or whether this mechanism could affect exercise tolerance in patients with chronic heart failure.

Minotti et al. (1992) measured amplitude and area of the M wave (compound muscle action potential) decline during progressive, fatiguing exercise in an attempt to determine whether the accelerated fatigue in these patients is due to impaired muscle activation as a result of inadequate central motor drive or neuromuscular transmission, or by a change in muscle itself. Their findings indicate that the more rapid onset of muscle fatigue in patients with congestive heart failure is not caused by impaired central motor drive or neuromuscular junction transmission. Rather, they suggest that this fatigue is caused by an abnormality in the muscle itself.

IV. Additional factors and their possible effect on exercise performance in patients with chronic heart failure

It is possible that the medication ingested by patients with chronic heart failure, chronic deconditioning due to heart failure, or the age of the patient might also contribute to the impaired exercise performance of these patients.

(1) Effects of medication on exercise performance in patients with heart failure

Ingestion of medication plays a key role in the management of symptoms in patients with chronic heart failure and the effect of these agents on exercise performance requires consideration. A recent significant development in medical management of heart failure is the introduction of angiotensin converting enzyme (ACE) inhibitors. Chronic ingestion of either captopril and enalapril not only improves signs and symptoms of heart failure and increase exercise performance, but reduces mortality in patients with chronic heart failure (SOLVD Investigators 1992; Pfeffer et al. 1992).

The negative inotropic effect of beta blockers causes haemodynamic and clinical deterioration in patients with heart failure (Kupper 1991), and may also cause an increased rate of perceived exertion during submaximal exercise effecting either the central nervous system or skeletal muscle in these patients (Derman et al. 1991). Diuretics are effective in reducing total body sodium and water content, and thus relieving the symptoms due to fluid retention in patients with heart failure, but there are no data to suggest that they improve quality of life or exercise tolerance in these patients. Digitalis does appear to be effective in improving prognosis in patients with heart failure (Packer 1992), but the benefits are confined to those patients who have markedly dilated left ventricles, with markedly impaired systolic function.

(2) Physical deconditioning

Patients with heart failure are less active than normal healthy controls. The pattern of skeletal muscle changes seen in patients with chronic heart failure is consistent with the effects of chronic exercise deconditioning, but the magnitude of these changes exceeds those which occur after exercise deconditioning in normal subjects (Coyle et al. 1984). In both states there is exercise intolerance, sympathetic activation, increased resting heart rate, reduced heart rate variability, atrophied skeletal muscle and reduced activity of skeletal muscle oxidative enzymes (Adamopoulos & Coats 1991; Saltin et al. 1988; Sullivan et al. 1986; Drexler et al. 1987b). Deconditioning appears to be one factor involved in the development of these potentially reversible skeletal muscle alterations (Drexler et al. 1992) and for the impaired peripheral vasodilation during exercise in patients with chronic heart failure (Sinoway et al. 1988).

(3) Contribution of age to exercise intolerance in patients with heart failure

As various physiological changes occur with increasing age, it is important that these changes are considered when the physiological response to exercise in patients with heart failure is gauged. Although it has been reported that oxygen consumption, cardiac output, stroke volume, ejection fraction, heart rate and Frank-Starling response at maximal exercise are significantly reduced in normal elderly subjects compared to younger subjects (Kitzman et al. 1989), the muscle strength of patients with chronic heart failure (Buller et al. 1991) is significantly less than age-matched, sedentary controls (Minotti et al. 1991a). Hence, while cardiovascular function is attenuated in both elderly persons and elderly persons with chronic heart failure, skeletal muscle strength appears to be affected to a greater extent in patients with chronic heart failure than in age-matched sedentary controls.

V. Effects of exercise training on exercise performance in patients with heart failure.

The effects of exercise training in patients with heart failure have previously been reviewed. Briefly, exercise training programmes are of benefit in improving exercise tolerance and symptoms of patients with moderate to severe heart failure secondary to ischaemic heart disease (Conn et al. 1982; Sylvan et al. 1988; Kellerman et al. 1988). Minotti et al. (1990) showed that regular exercise training improved the endurance and metabolism of trained muscle but had no effect on untrained forearm muscles. Other adaptations to exercise training which may occur are: decreased adrenergic activity during exercise (Coats et al. 1990), changes in substrate delivery, and alterations in intramuscular blood flow distribution (Arnolda et al. 1990). Increased exercise performance in these patients is associated with improvements in oxygen uptake by the exercising muscle (Sullivan et al. 1988a; Coats et al. 1990).

Recently Stratton et al. (1994) reported a 65% increase in endurance exercise capacity, reduced rates of intracellular acidification and PCr utilization during exercise, and an increased maximal rate of mitochondrial ATP synthesis after one month of forearm isotonic and isometric exercise training in patients with heart failure.

VI. Overall summary and aim of this thesis

According to this literature review, heart failure is probably more appropriately defined as a clinical condition in which a chronic cardiac abnormality results in effort intolerance and eventually a series

of neurohumoral adaptations (Packer 1988). Previously, central cardiorespiratory factors were the main focus of researchers, who measured mainly central physiological variables during exercise to determine the limitation of exercise in these patients. However, more recently exercise intolerance in patients with CHF has been explained by abnormalities in peripheral blood flow and in skeletal muscle metabolism

The many inconsistencies discussed in the review indicate that other factors could be responsible for exercise intolerance in these patients. It is probable that whilst both central and peripheral factors contribute to the exercise intolerance in these patients, patients may be limited more by disease of skeletal muscle than by dyspnoea or central fatigue. However, few studies have examined the role of skeletal muscle in the limitation of exercise performance in patients with chronic heart failure.

Therefore the first aim of Chapter 3 is to measure both isometric and isokinetic peak skeletal muscle function, as well as tests of skeletal muscle function during repeated skeletal muscle contractions and time to the onset of fatigue and isokinetic total work of the lower limb muscles. In addition, few studies have measured physiological variables when patients and controls have started at low identical workloads. Thus Chapter 3 also describes the physiological response during graded exercise to exhaustion in patients with severe CHF and normal controls using identical exercise test protocols.

Some recent studies have documented gross changes in the histology of the skeletal muscles in patients with CHF but it is not known if the severity of the histological abnormalities of the skeletal muscles in patients with CHF relates to the exercise intolerance experienced by these patients. Furthermore, no studies have provided control groups, few studies have examined the ultrastructure of the skeletal muscle in patients with CHF, and a method for quantification of skeletal muscle structural damage does not exist. Thus the aim of Chapter 4 was to focus on the structural changes of the skeletal muscle in patients with severe chronic heart failure and to describe the histological and ultrastructural findings in skeletal muscle biopsy samples from patients with CHF, to develop a simple histological grading system to quantify the magnitude of these changes, and to determine the relationship of these changes to alterations in the exercise capacity of these patients. The conclusions from these studies are detailed in Chapter 5.

Chapter 2 describes the methodology used in this thesis, whilst Chapter 6 lists the references for this thesis. Further research of the peripheral alterations and abnormalities of skeletal muscle structure and function in these patients is warranted.

CHAPTER 2

GENERAL METHODOLOGY

MATERIALS AND METHODS:

The general methodology for chapters 3 and 4 are described in this chapter.

(1) General considerations

To be included in the studies, patients had to be free of any serious respiratory or musculo-skeletal disorders. In addition, the control subjects had to be free of any underlying cardiovascular disease. Subjects and controls were instructed to avoid any strenuous physical exercise for 36 hours preceding each laboratory test. No caffeine containing food or drinks were to be consumed on the day of testing. All tests were performed at the same time of day, approximately two to three hours after ingestion of the last medication dose. The study protocols were approved by the Ethics and Research Committee of the Faculty of Medicine at the University of Cape Town. All subjects provided written informed consent and were given a full medical examination and resting electrocardiogram before the study commenced.

(2) Anthropometrical analysis of body composition

Body composition was assessed using an anthropometric technique which fractionated the body composition into muscle mass (Martin et al. 1990) and fat and lean body mass (Durnin and Womersley 1974). Chest measurements and relaxed and contracted arm girth measurements were also recorded. Somatotype was calculated according to the procedure of Heath and Carter (1967). Standing height without shoes was measured to the nearest 0.1 cm. Skinfold thickness was measured to the closest 0.1 mm at triceps, biceps, suprailliac and subscapula sites on the right side of the body with Holtain skinfold calipers. Density was estimated from the sum of the four skinfold thicknesses, using the equations of Durnin and Womersley (1974). Percent fat was calculated from density values using the Siri formula.

(3) Blood pressure and heart rate measurement at rest and during exercise

After subjects remained seated in a quiet environment for a period of fifteen minutes, the resting heart rate and blood pressure (Korotkoff Phase I and IV) was measured and recorded. Blood pressure at rest and every two minutes during exercise was measured by means of audible sphygmomanometry using a calibrated mercury column sphygmomanometer with an appropriately sized cuff. The cuff was placed on the arm that did not have an intravenous cannula. The measurement of diastolic blood pressure during exercise is sometimes difficult. However, if Phase IV of the Korotkoff sounds is taken, the results are quite reproducible. Heart rate was determined at rest and each minute during exercise, using a Loheimer M607 monitor (Munich, Germany) using self-adhering electrodes placed in the CM5 position.

(4) Graded exercise tests to exhaustion

Both patients and control subjects performed maximal progressive exercise to exhaustion on an electro-mechanically braked cycle ergometer (Godart, Bilthoven, Holland). Prior to testing the height of the saddle was adjusted to the preferred height for the patient. During exercise, subjects cycled with a model no. 2766 counterbalanced head support holding a model no. 2700 Rudolph valve (both by Hans Rudolph Inc., Kansas City, KA). A nasal clip prevented nasal breathing. Subjects commenced pedaling at a workload of 5 watts (W) for the first three minutes. The workload was increased by 10 W/min until the subject voluntarily terminated the test. During exercise expired air was analyzed for O_2 (Ametec O_2 Analyzer Model 3A, Theridox, Pittsburg, USA) and CO_2 content (Ametec Carbon Dioxide Analyzer Model CD-3A, Theridox, Pittsburg, USA). Both analyzers were calibrated before and after each test using gases of known composition. Inspiratory volume was recorded with a Mijnhardt dry gas meter which had been calibrated against a Collins chain-compensated gasometer (Collins Inc., Braintree, Massachusetts, USA).

The VO_2 max was taken as the highest rate of oxygen consumption measured during any 60 seconds (Noakes 1988). Rates of oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER) and ventilation (V_i) were calculated every 3 seconds by an on-line micro computer (Sperry, Salt Lake City, Utah, USA) using software (ART) based on conventional equations as previously described from this laboratory (Noakes et al. 1990). The average value for each minute for each parameter was stored for later printing.

Peak workload was taken as the highest workload maintained for a complete minute during the test. When a subject was unable to complete the full minute at a particular workload, the workload of the immediately preceding, completed workload was recorded as the peak workload. The same method also was used to calculate the exercise time to exhaustion.

Patients and control subjects were asked to report their level of perceived exertion from the appropriate scale after each minute of the exercise test. Most studies have used the 20-point Borg scale. This method caused inaccurate scaling of variables such as blood lactate concentrations and ventilation, which increase according to non-linear power functions (Noble et al. 1982). Consequently we have used the 10-point category scale with ratio properties (Borg 1980, 1982). The ratio scale has verbal expressions which are simple to understand and more accurately describe sensations of perceived peripheral effort.

* Indications or halting the exercise test before voluntary exhaustion were:

(a) Adverse symptoms; for example, severe dyspnea, light headedness, confusion and severe fatigue

(b) Adverse signs; for example, facial pallor, drop in heart rate or blood pressure, or failure of blood pressure to rise with increasing effort, systolic blood pressure exceeding 280 mmHg, or diastolic blood pressure exceeding 140 mmHg.

(5) Collection of blood for analysis of blood lactate concentration during graded exercise to exhaustion

Prior to the start of the cycle test, an 18-gauge flexible catheter (Criticon, Tampa, Florida, USA) was inserted into a subcutaneous forearm vein of each subject. A 3-way stopcock (Discofix-3, Belgium) was attached for multiple blood sampling and kept patent by means of a saline drip (Sabax sodium chloride 0.9% / Plexitron AFC0197, Johannesburg, South Africa). In cases where blood sampling was difficult, a blood pressure cuff was applied to increase distal venous pressure. Before blood samples were collected, 2.0 mls of blood were drawn to remove any saline that may have been present in the catheter. Blood sampling took place in the rested state before exercise began, and subsequently at one-minute intervals during the exercise bout. A further blood sample

was drawn at exhaustion and again each minute for 5 minutes into the post-exercise recovery period. On completion, the samples were spun in a Sigma 302-K centrifuge (Munich, Germany) at 2700 RPM for 12 minutes. Thereafter, the plasma was removed and frozen until later analysis for lactate concentration.

(6) Analysis of blood lactate concentrations

This method was adapted from Gutman and Wahlefeld (1974). Plastic Greiner test-tubes containing 2 ml of 0.6 N perchloric acid (PCA) were weighed on a Sartorius 1412 scale (Gottingen, West Germany) and the weights were recorded. These tubes were kept in a refrigerator until the samples were ready to be collected, at which time the tubes were then kept cold on ice. Approximately 1 ml of whole blood sample was added to the tube containing the PCA, and inverted vigorously to mix. The samples were kept on ice until completion of the exercise test, and then centrifuged at 4°C for 15 minutes at 2000 rpm. They were then re-weighed to calculate the actual volume of blood added. The supernatant was then decanted into labeled Eppendorf test-tubes, and stored at -20°C until the assay was performed.

Assay method: Glass test-tubes were labeled as follows; 3 x blanks, 2 x standards, and then sample tubes in duplicate. Reagents were added to each of the tubes as follows:

| | Standard (ml) | Blank (ml) | Sample tube (ml) |
|------------------|---------------|------------|------------------|
| Hydrazine buffer | 1 | 1 | 1 |
| NAD | 0.1 | 0.1 | 0.1 |
| PAC | | 0.1 | |
| LDH | 0.1 | 0.1 | 0.1 |
| Supernatant | | | 0.1 |
| Standard | 0.1 | | |

The hydrazine/ glycine buffer was 0.4M hydrazine and 0.5M glycine in distilled H₂O, adjusted to pH 9.0. The NAD solution (\pm 30mM) was prepared by dissolving 100 mg NAD in 5 ml distilled H₂O, and then stored at -20°C until needed. The LDH suspension (\pm 5 mg protein/ml) was stored at 4°C and used undiluted. A dilution of 1.0M lactate standard was used.

The standard, blank and test solutions were separately mixed on a vortex mixer. These solutions were covered with parafilm, and then placed in an incubator to react for 30 minutes at 37°C. Optical density changes were recorded with a Beckman DU-62 spectrophotometer (Beckman Instruments Inc., England) previously zeroed at 340 nm against distilled H₂O. The following calculation was used to determine the lactate concentration of the samples :

$$\text{Lactate conc. (mmol/l)} = \frac{\text{AB} \times 1.945 \times (2 + \text{vol blood})}{\text{vol blood}}$$

(Where AB = sample absorbance - blank absorbance)

(7) Skeletal muscle biopsy procedure

Ten minutes after the patient had completed the graded exercise test, a sample of muscle was removed from the vastus lateralis under local anaesthesia according to the needle biopsy technique of Bergstrom et al. (1962), as modified by Evans et al. (1982).

The muscle was divided into two pieces: One piece of the muscle was snap-frozen in isopentane and cooled in liquid nitrogen. Transverse sections (5-7 μm) were stained for haemotoxylin and eosin, Gomori's trichrome, β -nicotinamide adenine dinucleotide reduced (NADH), Oil Red O and adenosine triphosphate (ATPase) at pH 4.3, 4.6, and 9.4. The fibre type proportions and the minimum diameter of approximately 150 Type I and Type II muscle fibres differentiated by ATPase staining were measured using an IBAS Kontron image analysis system.

The second portion of the muscle was desensitized in 3% phosphate buffered glutaraldehyde for 10 mins. The tissue was sliced into smaller sections and fixed overnight. The slivers of transverse and longitudinal muscle sections were post-fixed in 1% buffered osmium tetroxide (pH 7.4) and processed by standard methods into Spurr's epoxy resin. Ultra-thin transverse and longitudinal sections were stained with uranyl acetate and lead citrate and examined using a Phillips 201 electron microscope.

Ultrastructural morphometric measurements of the capillary basement membranes were performed using the technique of Spierstein et al. (1968) and an IBAS Kontron Image analysis

system. Only transverse capillaries were selected for analysis. A plastic sheet with 20 radiating lines exactly 18 degrees apart, was placed centrally on the capillary. Measurements were made at the points where the lines intersected the basement membrane. Measurements were not taken when the radiating lines intersected a pericyte. If at least 10 measurements could not be obtained from a single capillary, it was discarded.

(8) Tests of skeletal muscle function

(i) Isometric muscle function

Maximum isometric voluntary strength of the right quadriceps muscle was measured on a custom-made, leg stabilizing chair and load cell built according to the specifications of Edwards et al. (1977). In that chair, the patient sat with his or her arms folded, back angled at 90°, knees at 90° flexion and pelvis secured to the chair by an adjustable belt. Once the use of the hip flexors had been limited by securing the pelvis, a cuff was placed above the malleoli of the right ankle and linked via a chain to a pre-calibrated strain gauge for the measurement of torque during contraction of the knee extensors. The lever length, used to calculate torque, was the distance between the attachment of the chain to the cuff and the lateral epicondyle of the knee.

Outputs from the strain gauge were processed by a locally built Data Acquisition Unit (Scientific Exercise Systems, Cape Town) interfaced with an IBM-compatible personal computer, which displayed the results on the computer screen. Patients first performed three 4 sec maximum voluntary contractions (MVCs) for the measurement of peak isometric torque. Sixty seconds after completion of this test the subject was encouraged to generate a MVC for six seconds followed by a four second rest period. Throughout this test, the patients were encouraged verbally to produce maximal contractions and to repeat the cycle as frequently as possible. This cycle was repeated until the subject was unable to generate a torque of 70% of their initial MVC. The time to reach this point was recorded as the time to fatigue, and is a measure of resistance of the skeletal muscle to the development of fatigue (Bigland-Richie et al. 1986).

(ii) Isokinetic muscle function

Isokinetic muscle strength was measured with a Cybex Isokinetic Dynamometer (Cybex II Isokinetic Dynamometer; Cybex, Ronkonkoma, NY) with a Cybex Data Reduction computer. This system is designed to maintain angular velocity, constant at a pre-set level regardless of the torque generated by the subject. The subjects were seated on the Cybex apparatus with the head of the femur fully supported and the knee flexed to 90°. The lever arm was secured to the subject via a shin pad placed just proximal to the malleoli. In order to isolate movement to the knee joint, thigh and shin pad straps were fastened as tight as were tolerable for the patient. During the testing, subjects were instructed to keep their arms folded across their chests. The fulcrum of the lever was positioned in line with the axis of rotation which corresponds to the transverse line through the femoral condyles. Each patient was given a practice bout at each testing speed. This warm-up consisted of 5 familiarization repetitions at 60°/sec and at 180°/sec. After the familiarization period, patients rested for 2 minutes before starting the test. Any necessary adjustments to the equipment were made during this time. The strength test measured maximum isokinetic torque of the quadriceps and the hamstring muscle groups of the dominant limb, recorded during 3 maximal contractions through a full range of motion at a limb contraction speed of 60°/sec. An endurance test of the dominant leg was then performed. After a 5 minute rest, the subject performed repeated full knee extensions for 25 seconds at a rate of 180°/sec. Total work and power generated by the quadriceps and hamstrings as well as the peak acceleration energy during the first 0.125 seconds of torque production at this speed were recorded. Verbal encouragement was given throughout the tests.

CHAPTER 3

EXERCISE TOLERANCE AND SKELETAL MUSCLE FUNCTION IN PATIENTS WITH CHRONIC HEART FAILURE

Introduction

As described in Chapter 1, fatigue and exercise intolerance are amongst the most common symptoms experienced by patients with CHF.

Abnormalities in peripheral blood flow (Zelis et al. 1974; Zelis and Flaim 1982; Le Jemtel et al. 1986; Sullivan et al. 1989) and in skeletal muscle metabolism (Wiener et al. 1986; Massie et al. 1987a, b; Sullivan et al. 1990, 1991; Drexler et al. 1988, 1992; Mancini et al. 1992; 1994) are present in patients with CHF and could contribute to their impaired exercise tolerance. The metabolic abnormalities and the impaired exercise tolerance may be present even without alteration in limb blood flow (Wiener et al. 1986; Massie et al. 1988) and may be partially reversed by exercise training (Stratton et al. 1994). It is therefore important to study the skeletal muscle itself to gain understanding of exercise intolerance in patients with CHF.

Historically, it has been argued that pulmonary congestion due to left heart failure and reduced lung compliance cause dyspnoea that limits the exercise tolerance of such patients. Thus central cardiorespiratory factors were the main focus of researchers, who measured mainly central physiological variables during exercise to determine the limitation of exercise in these patients (Clausen 1976, Bruce et al. 1977, Reddy et al. 1988, Cowley et al. 1991, Kitzman et al. 1991, Myers et al. 1992).

Recent studies however, have indicated that exercise capacity in patients with CHF may not be limited solely by central cardiorespiratory factors (Franciosa et al. 1981, 1984; Sullivan et al. 1989; Roubin et al. 1990; Minotti et al. 1992; Jondeau et al. 1992). Rather, the focus of more recent studies has shifted to aspects of the peripheral circulation and skeletal muscle as possible factors limiting the exercise tolerance of patients with CHF.

However, only few studies have measured skeletal muscle function during exercise (Lipkin et al. 1988; Minotti et al. 1991; Buller et al. 1991; Magnusson et al. 1994) and some have drawn conclusions from tests of single maximal skeletal muscle contractions.

Therefore the first aim of this study was to measure both isometric and isokinetic peak skeletal muscle function, as well as tests of skeletal muscle function during repeated skeletal muscle contractions and time to the onset of fatigue of the lower limb muscles.

Most studies of graded exercise to exhaustion in patients with CHF have examined physiological variables during exercise in both patients with CHF and normal controls. However, both patients and controls initiate exercise at different workrates, and conclusions about comparative exercise physiology are drawn from measurements at relative percentages of the VO_2 max (Colucci et al. 1989, Riley et al. 1990). Few studies however have measured physiological variables when patients and controls have started at low identical workloads. Thus the second aim of this study was to describe the physiological response during graded exercise to exhaustion in patients with severe CHF and normal controls using identical exercise protocols.

Methodology

Subjects characteristics on entry to the study

Table 3.1 Details the characteristics of the patients with CHF on entry to the study.

Table 3.1 Characteristics of patients with CHF

| Patient | Sex | Age (yr) | Mass (kg) | Duration of heart failure (months) | Cause of cardiac failure | Resting EF% | Hb (g%) |
|-------------|-----|-----------|-----------|------------------------------------|--------------------------|-------------|-------------|
| 1 | M | 60 | 71 | 12 | Ischaemic CMO | 12 | 13.3 |
| 2 | M | 42 | 63 | 3 | Post Viral CMO | 18 | 14.0 |
| 3 | M | 40 | 68 | 12 | Idiopathic CMO | 12 | 14.6 |
| 4 | M | 46 | 65 | 6 | Ischaemic CMO | 12 | 14.2 |
| 5 | M | 47 | 69 | 9 | Ischaemic CMO | 15 | 12.7 |
| 6 | M | 52 | 85 | 6 | Idiopathic CMO | 18 | 15.5 |
| 7 | M | 55 | 97 | 15 | Ischaemic CMO | 20 | 12.6 |
| 8 | M | 45 | 120 | 12 | Idiopathic CMO | 26 | 13.1 |
| 9 | M | 59 | 80 | 20 | Idiopathic CMO | 13 | 11.2 |
| 10 | M | 34 | 106 | 24 | Ischaemic CMO | 30 | 14.1 |
| 11 | M | 36 | 113 | 24 | Ischaemic CMO | 14 | 13.6 |
| Mean | | 47 | 85 | 13 | | 17 | 13.5 |
| SEM | | 3 | 6 | 2 | | 2 | 0.3 |

Abbreviations: M, male; m, metres; kg, kilogram; CMO, cardiomyopathy; EF, ejection fraction; Hb, haemoglobin; SEM, standard error of the mean.

Eleven male patients mean (\pm SEM) age 47 ± 3 yr (range 34-60 yr), mean mass 85.0 ± 6.0 kg (range 63-120 kg) with CHF (NYHA Class III-IV) who were awaiting heart transplantation were recruited from the Departments of Cardio-Thoracic Surgery and Cardiology at Groote Schuur Hospital, Cape Town, South Africa. Selection of the patients for cardiac transplantation was by internationally accepted criteria (Mudge et al. 1993). Four patients had CHF due to idiopathic

cardiomyopathy, six due to ischaemic cardiomyopathy, and one due to viral myocarditis. Mean ejection fraction of the CHF group prior to cardiac transplantation was $17 \pm 2\%$ (range 12-30%). No patients had exercised regularly for at least 2 yr before commencement of the study. Patients were excluded from this study if they had signs, symptoms or a history of any other chronic disease, or if they were bedridden or unable to exercise. Data for isometric skeletal muscle function was not obtained from one patient who underwent heart transplantation before skeletal muscle function testing could be performed. Equipment malfunction interrupted respiratory gas testing in two control subjects. Blood lactate was not measured in one control subject.

Medications

Patients receiving medications at the start of the trial were instructed to continue with their medication during the period of exercise testing. Medications used by these patients are described in *Table 3.2*.

Table 3.2. List of medications ingested by patients with CHF

| Pt No. | Medication |
|---------------|---|
| 1. | Captopril; digoxin; furosemide; glibenclamide; isosorbide dinitrate; aspirin |
| 2. | Berotec; ephyllin; furosemide; digoxin; warfarin |
| 3. | Captopril; furosemide; digoxin; potassium supplementation |
| 4. | Captopril; digoxin; metolazone; warfarin; potassium supplementation |
| 5. | Captopril; digoxin; furosemide; spironolactone; metolazone; warfarin; potassium supplementation |
| 6. | Digoxin; furosemide; allopurinol; voltaren; colchicine; warfarin; potassium supplementation |
| 7. | Captopril; furosemide; allopurinol; zaroxyllin; warfarin; potassium supplementation |
| 8. | Captopril; furosemide; digoxin; disprin; warfarin |
| 9. | Captopril; digoxin; furosemide; isosorbide dinitrate; warfarin; magnesium and potassium supplementation |
| 10. | Enalapril; digoxin; furosemide; potassium supplementation |
| 11. | Captopril; diltiazem; digoxin; furosemide; ranitidine; alprazolam; potassium supplementation |

All patients were receiving four or more medications. Categories of medications included diuretics, angiotensin converting enzyme inhibitors, digitalis, anticoagulants, allopurinol and potassium supplementation. Two patients were ingesting nitrates and one patient was ingesting oral anti-diabetic agents.

Exercise tests performed on these patients were compared to data from ten sedentary age- and mass- matched individuals who were free of any chronic disease. Mean age of this group was 39 ± 3 yr (range 26-56 yr), and average mass was 85.8 ± 5.3 kg (range 63-118 kg). These values were not significantly different from the patient group.

Exercise testing

Patients were tested on two occasions, one week apart, according to the methods detailed in Chapter 2. All tests were performed at the same time of day, two hours after the last dose of medication had been ingested. On the first day, patients underwent anthropometric assessment and performed a graded exercise test to exhaustion on a cycle ergometer for determination of peak oxygen consumption, heart rate and blood pressure responses and ratings of perceived exertion during exercise. Blood sampling for measurement of blood lactate concentration was performed during exercise.

During the second testing session, patients performed tests of isometric and isokinetic skeletal muscle function as detailed in Chapter 2. At least one hour was allowed for patients to recover between exercise bouts. Equipment malfunction interrupted respiratory gas testing in two control subjects.

Radionuclide angiocardigraphy for determination of resting left ventricular ejection fraction was performed only in the patients with CHF one week prior to the exercise test, according to conventional techniques.

Statistical analysis

All data are expressed as mean \pm SEM. The significance of differences between experimental variables of the two groups were analyzed using a Student's unpaired t-test with two-tailed p values. Statistical significance was established at the 0.05 and 0.01 confidence levels (Glanz 1980).

Results

Anthropometrical Analysis

Anthropometrical data are detailed in *Table 3.3*. Both the patient and the control groups displayed predominantly mesomorphic somatotypes. Waist-hip ratio, % body fat, lean body mass and anthropometrical estimation of muscle mass were not significantly different between groups. However, lean thigh volume (LTV) was significantly lower in the patient group compared to the control group ($p < 0.05$).

Table 3.3 Anthropometrical data displaying somatotype and morphometry

| Patient | Endo | Meso | Ecto | WHR | LTV (cc) | % Body Fat | LBM (kg) | AEMM (kg) | Ht (m) | Wt (kg) |
|----------------|------------|------------|------------|-------------|--------------|-------------|-------------|-------------|-------------|-----------|
| 1 | 4.5 | 3.3 | 2.9 | 0.94 | 2886 | 26.0 | 52.5 | 29.7 | 1.77 | 71 |
| 2 | 1.6 | 1.7 | 4.2 | 0.97 | 3164 | 13.0 | 54.9 | 27.9 | 1.77 | 63 |
| 3 | 4.5 | 5.9 | 0.0 | 0.90 | 3725 | 23.8 | 51.8 | 33.5 | 1.58 | 68 |
| 4 | 5.7 | 3.6 | 1.6 | 0.90 | 2326 | 27.2 | 47.1 | 25.1 | 1.65 | 65 |
| 5 | 3.1 | 4.1 | 0.1 | 0.93 | 2318 | 20.5 | 55.6 | 28.1 | 1.77 | 69 |
| 6 | 3.9 | 4.1 | 2.1 | 0.99 | 3981 | 23.6 | 64.9 | 39.4 | 1.83 | 85 |
| 7 | 5.3 | 5.9 | 0.1 | 1.03 | 4052 | 28.5 | 69.0 | 41.5 | 1.78 | 97 |
| 8 | 8.4 | 9.0 | 0.1 | 0.94 | 3499 | 35.8 | 77.0 | 44.4 | 1.74 | 120 |
| 9 | 2.6 | 5.1 | 0.3 | 1.16 | 3098 | 18.1 | 71.7 | 36.0 | 1.74 | 80 |
| 10 | 6.7 | 6.2 | 0.5 | 0.96 | 2893 | 26.6 | 77.8 | 34.6 | 1.87 | 106 |
| 11 | 6.2 | 7.3 | 0.1 | 1.08 | 3502 | 32.0 | 76.8 | 32.0 | 1.84 | 113 |
| Mean | 4.8 | 5.1 | 1.1 | 0.98 | 3222* | 25.0 | 63.6 | 33.8 | 1.74 | 85 |
| SEM | 0.6 | 0.6 | 0.4 | 0.02 | 171 | 1.8 | 3.3 | 1.8 | 0.9 | 6 |
| Control | | | | | | | | | | |
| 1 | 7.8 | 8.3 | -2.7 | 1.02 | 4456 | 33.9 | 79.4 | 45.2 | 1.74 | 118 |
| 2 | 1.7 | 3.3 | 4.6 | 0.79 | 4244 | 9.8 | 56.4 | 28.6 | 1.80 | 63 |
| 3 | 3.0 | 5.3 | 1.2 | 0.90 | 5429 | 20.5 | 63.6 | 38.5 | 1.76 | 80 |
| 4 | 3.8 | 4.8 | 1.0 | 0.89 | 4800 | 21.5 | 74.5 | 35.9 | 1.84 | 94 |
| 5 | 1.4 | 4.7 | 2.4 | 0.88 | 4764 | 14.0 | 64.5 | 37.6 | 1.79 | 75 |
| 6 | 4.7 | 5.9 | 0.1 | 1.03 | 4754 | 22.7 | 70.6 | 36.8 | 1.72 | 92 |
| 7 | 5.9 | 5.0 | 0.9 | 0.88 | 3818 | 24.0 | 64.6 | 37.6 | 1.81 | 102 |
| 8 | 3.8 | 4.7 | 0.2 | 0.97 | 2821 | 22.1 | 53.6 | 24.4 | 1.69 | 69 |
| 9 | 7.0 | 6.9 | 0.1 | 1.03 | 3348 | 31.0 | 70.0 | 35.7 | 1.84 | 85 |
| 10 | 4.7 | 8.3 | 1.3 | 0.95 | 4066 | 14.8 | 60.5 | 34.1 | 1.80 | 78 |
| Mean | 4.4 | 5.7 | 0.9 | 0.93 | 4250 | 21.4 | 65.8 | 35.4 | 1.78 | 86 |
| SEM | 0.7 | 0.5 | 0.6 | 0.03 | 244 | 2.3 | 2.5 | 2.4 | 1.55 | 5 |

Abbreviations: Endo, endomorphy; Meso, mesomorphy; Ecto, ectomorphy; WHR, waist hip ratio; LTV, lean thigh volume; cc cubic centilitres; LBM, lean body mass; kg, kilogram; AEMM, anthropometrical estimation of muscle mass; SEM, standard error of the mean. * = $p < 0.05$ CHF vs control subjects.

Resting cardiovascular measurements

Resting cardiovascular measurements are listed in *Table 3.4*. Resting heart rate (HR) in the CHF group was significantly higher than the control group ($p < 0.05$). Both resting systolic (SBP) and diastolic blood pressure (DBP) were significantly lower in the CHF group compared to the control group ($p < 0.05$).

Table 3.4 Resting cardiovascular measurements

| | HR (b/min) | SBP (mmHg) | DBP (mmHg) |
|----------------------|------------|------------|------------|
| Patient Group | 80 ± 3* | 111 ± 4* | 73 ± 4 * |
| Control Group | 70 ± 3 | 124 ± 2 | 82 ± 1 |

Abbreviations: HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; b/min, beats per min; mmHg, millimetres of mercury. * = $p < 0.05$ patients vs control subjects.

Exercise performance and cardiovascular and respiratory measurements during submaximal exercise

The exercise test protocol for both patient and control groups was identical (Chapter 2). Data are expressed at the same absolute workload (Figs 3.1,3,5,7,9,11,13) and at the same relative intensity of exercise: at 25, 50, 75, and 100 percent of peak oxygen consumption (%VO₂ peak; Figs 3.2,4,6,8,10,12,14).

Although HR tended to be higher at any given absolute workload during exercise in the patients compared to control subjects, it was only significantly higher at workloads of 5W and 80W ($p < 0.05$, Fig 3.1). HR was significantly increased compared to control subjects at 25% of VO₂ peak, ($p < 0.05$; Fig 3.2), but was lower than that of control subjects at 100% of VO₂ peak ($p < 0.05$; Fig 3.2). SBP was significantly lower in the patients compared to control subjects at workloads of 10, 20, 30, 40 and 50 W ($p < 0.05$), but not thereafter (Fig 3.3). However, SBP was significantly lower in the patients compared to control subjects at all relative intensities of exercise ($p < 0.05$; Fig 3.4).

Although oxygen consumption (VO₂) tended to be higher in the control group, it was not different between groups at any given absolute workload (Fig 3.5). However, patients had significantly lower values compared to control subjects at all relative exercise intensities ($p < 0.05$; Fig 3.6).

Minute ventilation (Vi) was not different between groups at any given workload (Fig. 3.7). However, Vi was significantly lower in the patients compared to the control group at any relative exercise intensity above 50 %VO₂ peak ($p < 0.001$; Fig 3.8).

Respiratory exchange ratio (RER) tended to be higher throughout exercise in the patients with chronic heart failure, and was significantly higher at 40, 50, 70, 80 and 90 W compared to the control subjects ($p < 0.05$; Fig. 3.9). At work intensities of 75 and 100 %VO₂ peak, RER was significantly lower in the patients compared to the control subjects ($p < 0.05$; Fig 3.10).

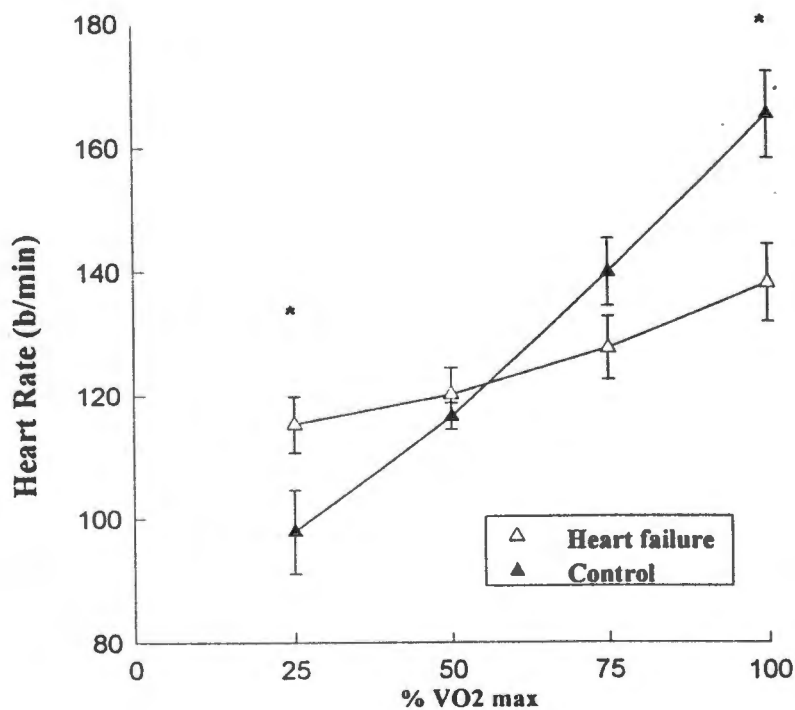
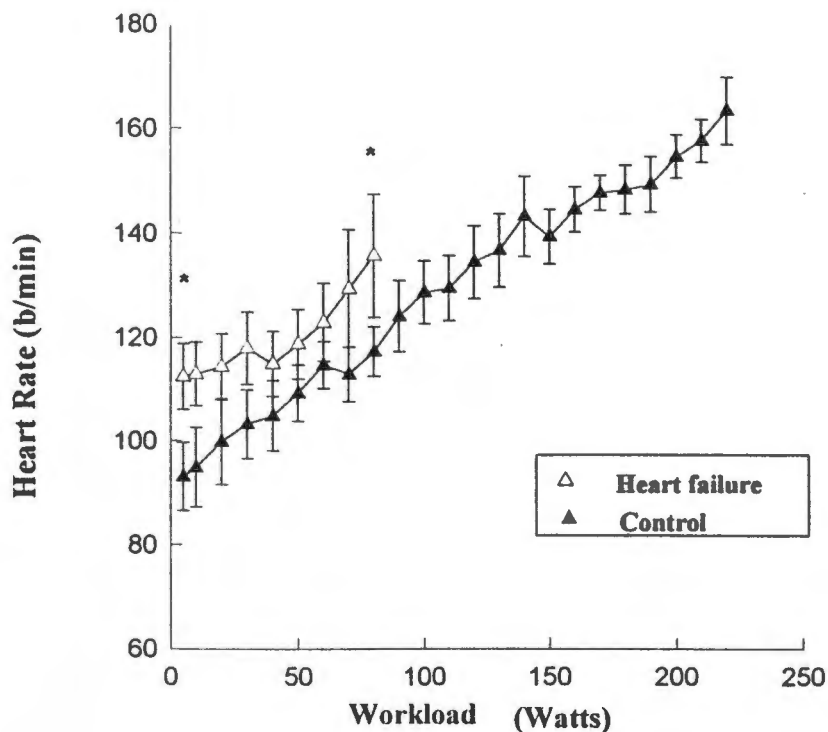


Fig 3.1-3.2. Heart rate responses depicted against increasing workload during graded exercise to exhaustion in patients with heart failure compared to controls. Data are expressed at the same absolute workload (Figs 3.1), and at the same relative intensity of exercise: 25, 50, 75, and 100 percent of maximum oxygen consumption (Figs 3.2). Data are displayed until > 50% of the patients and controls terminated the exercise test. Abbreviations: b/min, beats per minute; % VO₂ max, percent of the maximum of oxygen consumed in one minute. *= $p < 0.05$ heart failure vs control. All values are expressed as mean and standard error of the mean.

Fig 3.3

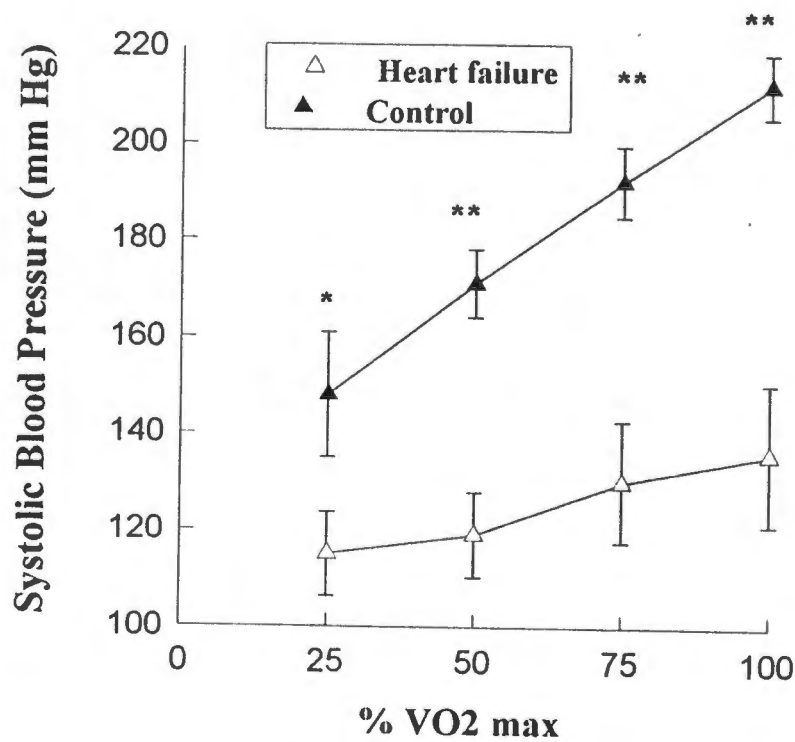
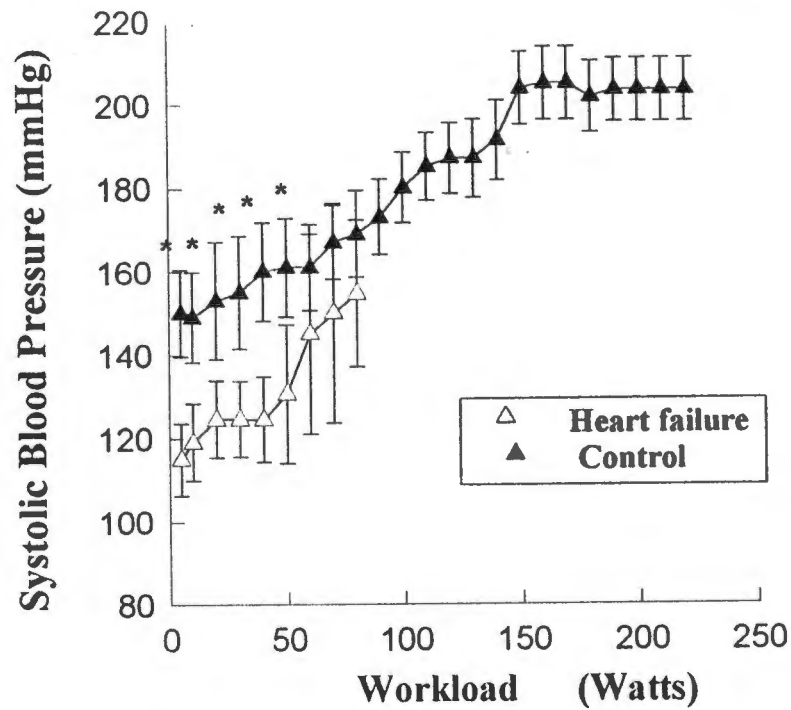


Fig 3.4

Fig 3.3-3.4. Systolic blood pressure responses depicted against increasing workload during graded exercise to exhaustion in patients with heart failure compared to controls. Data are expressed at the same absolute workload (Figs 3.3), and at the same relative intensity of exercise: 25, 50, 75, and 100 percent of maximum oxygen consumption (Figs 3.4). Data are displayed until > 50% of the patients and controls terminated the exercise test. Abbreviations: mmHg, millimeters of mercury; % VO₂ max, percent of the maximum of oxygen consumed in one minute. *= $p < 0.05$ **= $p < 0.01$ Heart failure vs Control. All values are expressed as mean and standard error of the mean.

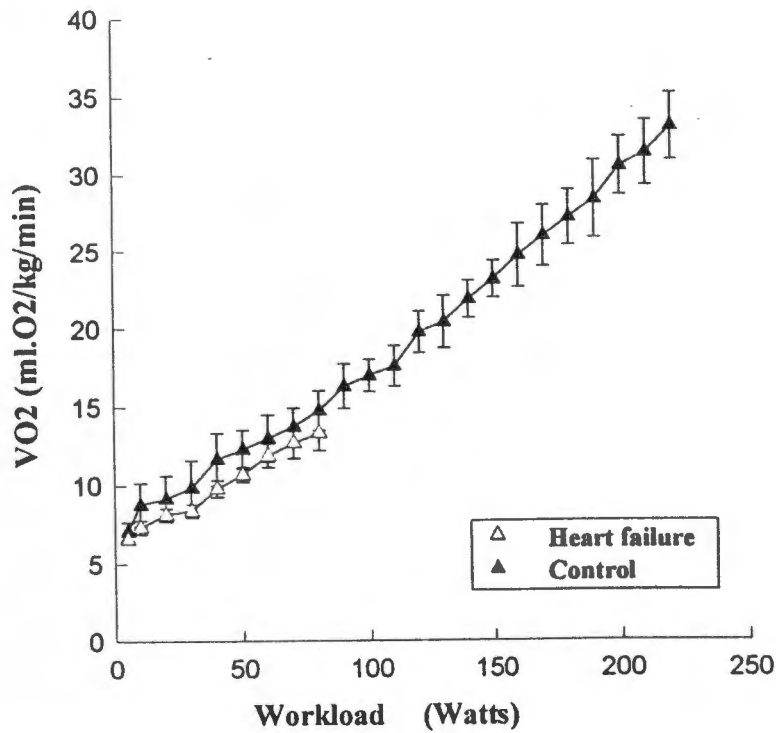


Fig 3.5

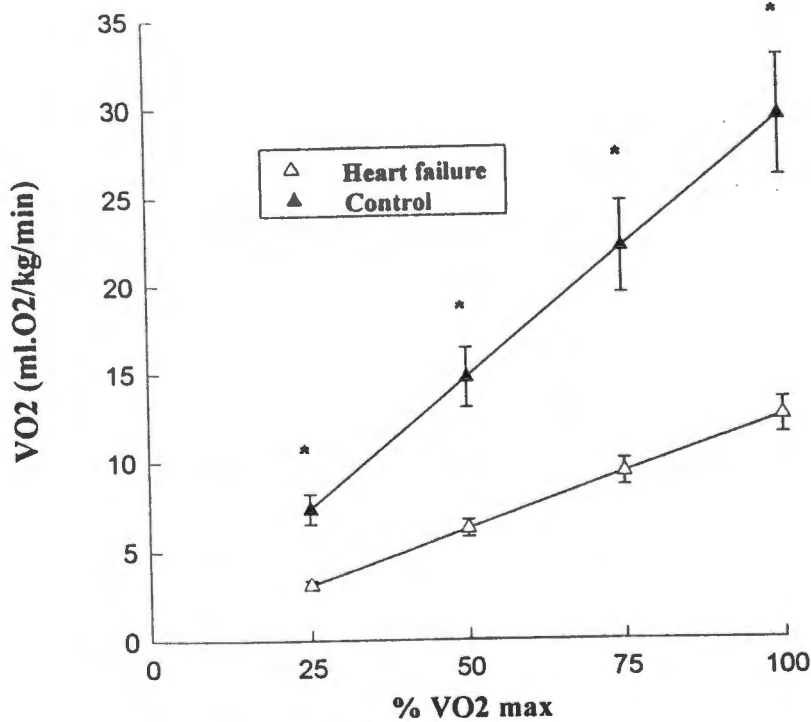


Fig 3.6

Fig 3.5-3.6. Oxygen consumption depicted against increasing workload during graded exercise to exhaustion in patients with heart failure compared to controls. Data are expressed at the same absolute workload, and at the same relative intensity of exercise: 25, 50, 75, and 100 percent of maximum oxygen consumption. Data are shown until > 50% of the subjects terminated the exercise test. Abbreviations: VO₂, volume of oxygen; ml.O₂/kg/min, millilitres of oxygen consumed per kilogram of body weight per minute; % VO₂ max, percent of the maximum of oxygen consumed in one minute. **p*<0.05 Heart failure vs Control. All values are expressed as mean and standard error of the mean.

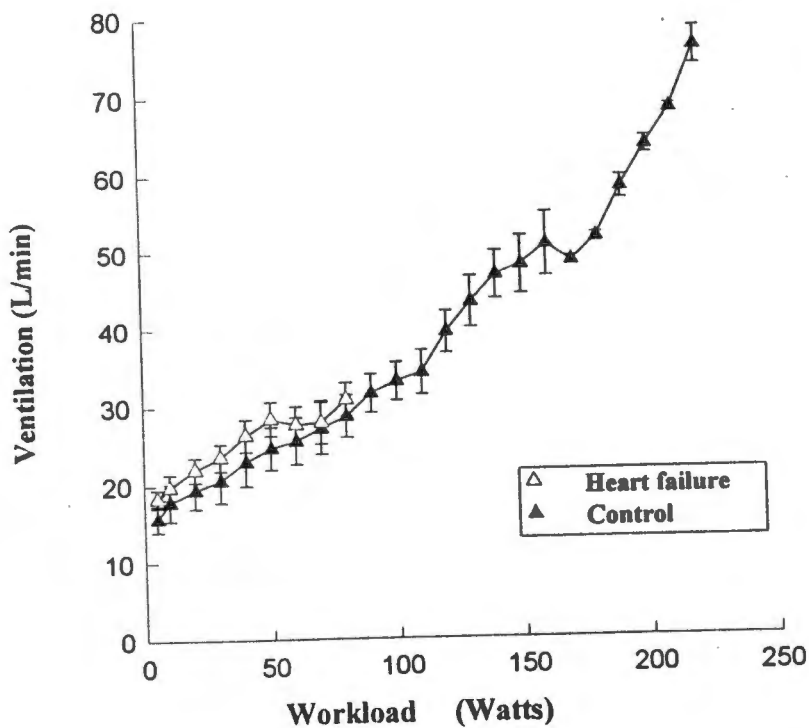


Fig 3.7

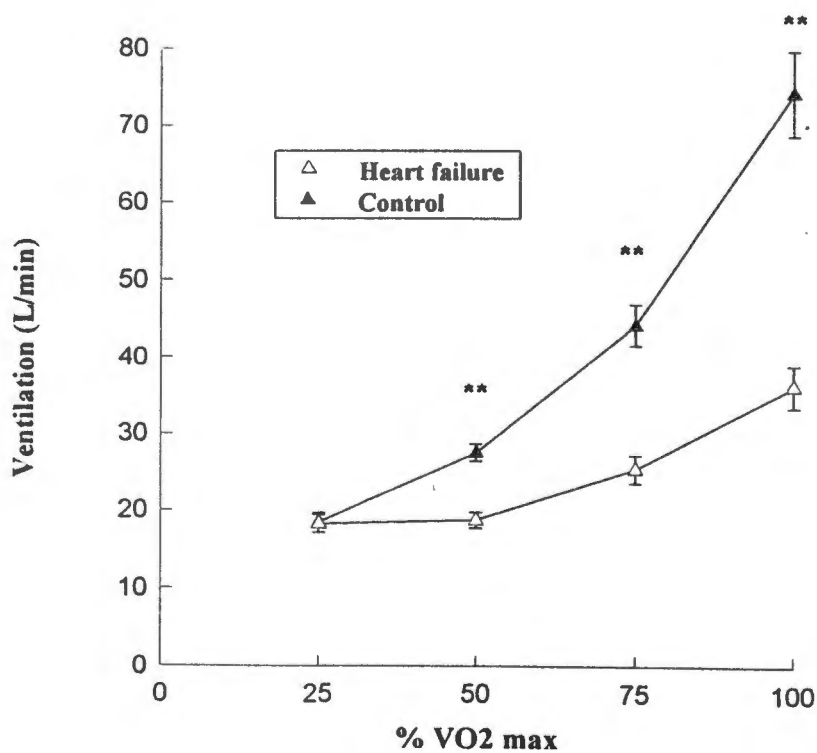


Fig 3.8

Fig 3.7-3.8. Ventilation depicted against increasing workload during graded exercise to exhaustion in patients with heart failure compared to controls. Data are expressed at the same absolute workload, and at the same relative intensity of exercise: 25, 50, 75, and 100 percent of maximum oxygen consumption. Data are displayed until > 50% of the patients and controls terminated the exercise test. Abbreviations: L/min, litres per minute; % VO₂ max, percent of the maximum volume of oxygen consumed in one minute. * $p < 0.05$ ** $p < 0.01$ Heart failure vs Control. All values are expressed as mean and standard error of the mean.

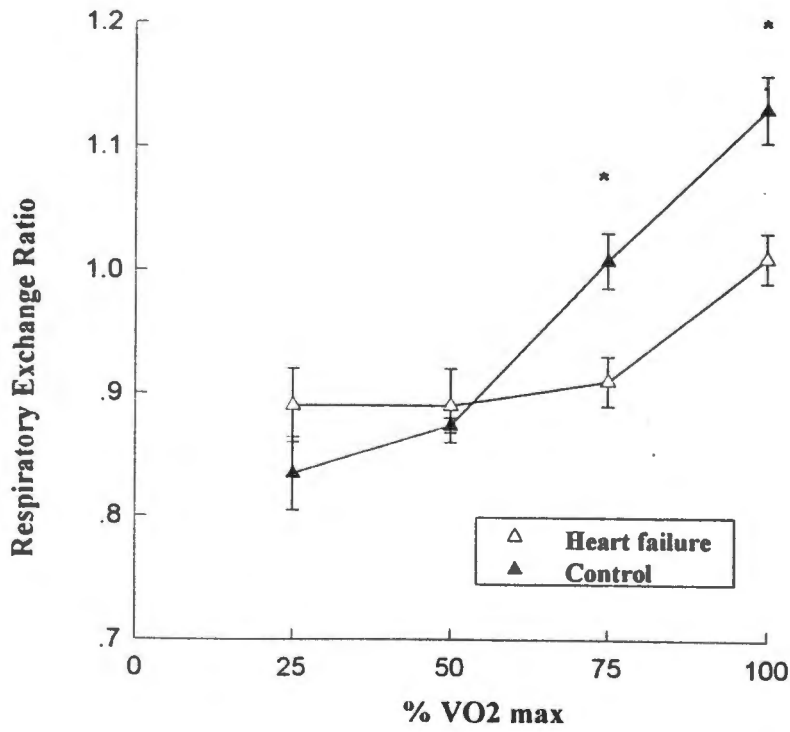
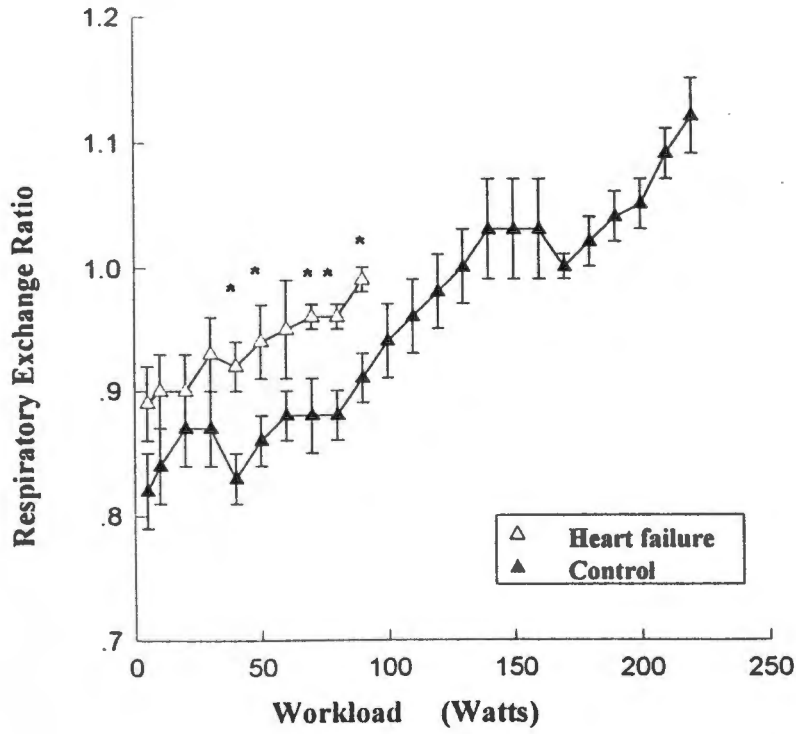


Fig 3.9-3.10. Respiratory exchange ratio depicted against increasing workload during graded exercise to exhaustion in patients with heart failure compared to controls. Data are expressed at the same absolute workload, and at the same relative intensity of exercise: 25, 50, 75, and 100 percent of maximum oxygen consumption. Data are shown until > 50% of the subjects terminated the exercise test. Abbreviations: % VO₂ max; percent of the maximum of oxygen consumed in one minute. * = p < 0.05 Heart failure vs Control. All values are expressed as mean and standard error of the mean.

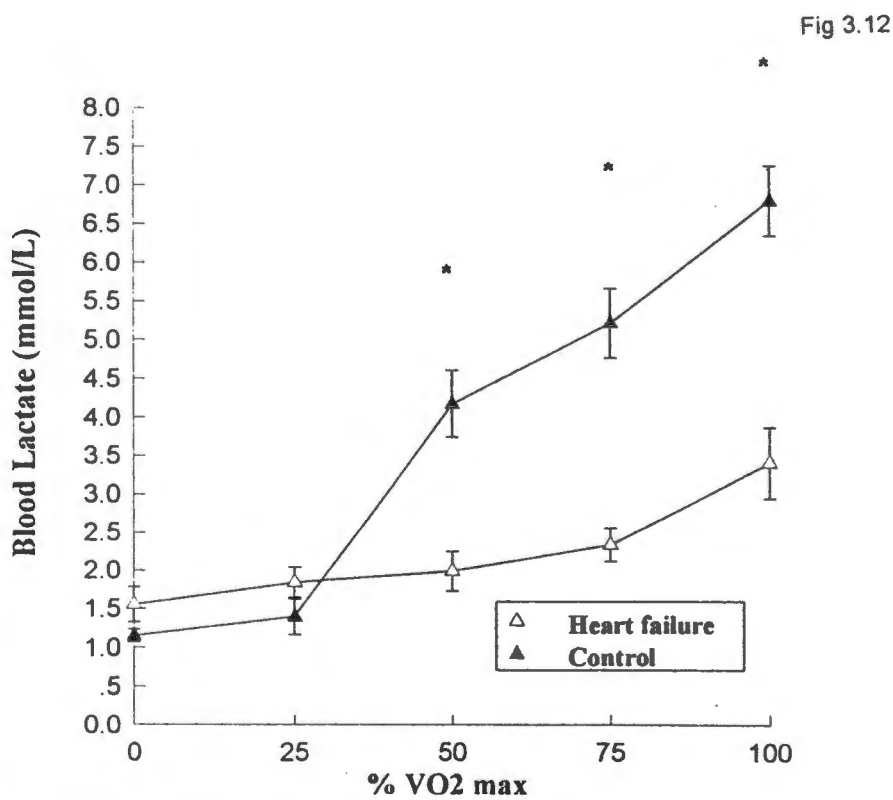
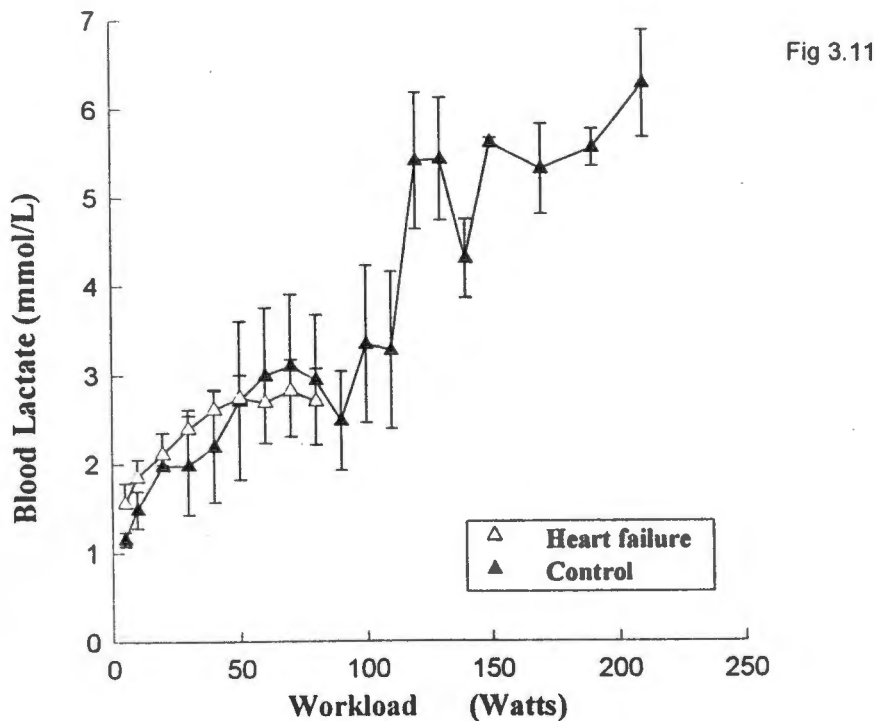


Fig 3.11-3.12. Lactate accumulation depicted against increasing workload during graded exercise to exhaustion in patients with heart failure compared to controls. Data are expressed at the same absolute workload, and at the same relative intensity of exercise: 25, 50, 75, and 100 percent of maximum oxygen consumption. Data are displayed until > 50% of the patients and controls terminated the exercise test. Abbreviations: mmol/L, millimole per litre; % VO₂ max, percent of the maximum volume of oxygen consumed in one minute. *= $p < 0.05$ **= $p < 0.01$ Heart failure vs Control. All values are expressed as mean and standard error of the mean.

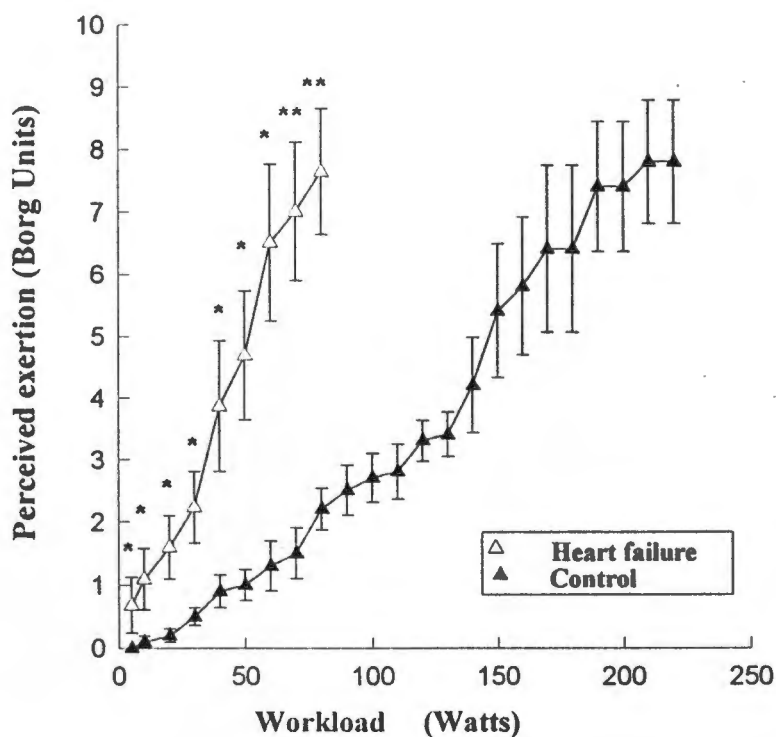


Fig 3.13

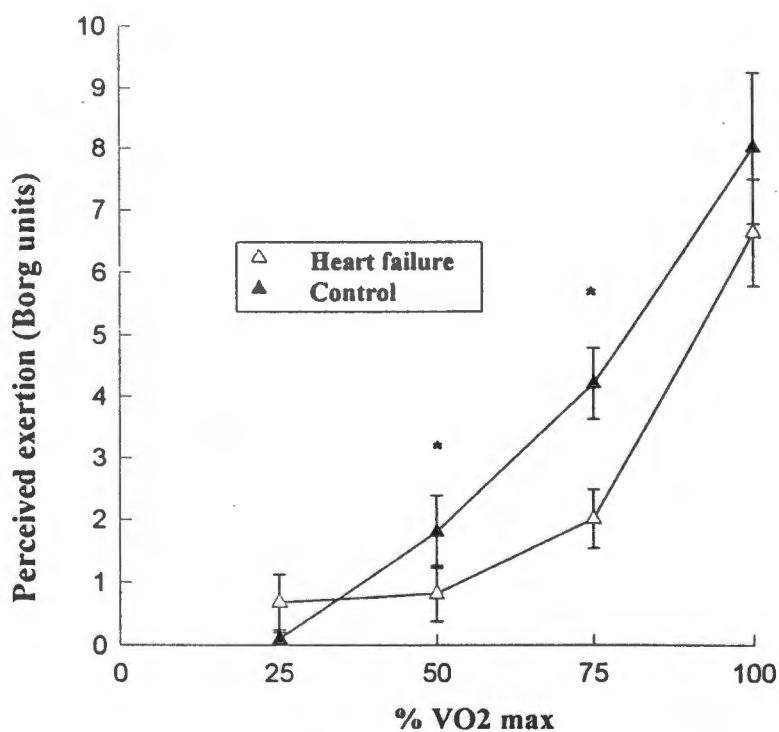


Fig 3.14

Fig 3.13-3.14. Rate of perceived exertion depicted against increasing workload during graded exercise to exhaustion in patients with heart failure compared to controls. Data are expressed at the same absolute workload, and at the same relative intensity of exercise: 25, 50, 75, and 100 percent of maximum oxygen consumption. Data are displayed until > 50% of the patients and controls terminated the exercise test. Abbreviations: % VO₂ max, percent of the maximum volume of oxygen consumed in one minute. *= $p < 0.05$ **= $p < 0.01$ Heart failure vs Control. All values are expressed as mean and standard error of the mean.

Although blood lactate concentrations were not different between groups at any given workload (Fig 3.11), concentrations were higher in the control group at exercise intensities above 50 % VO_2 peak ($p < 0.05$; Fig 3.12).

Ratings of perceived exertion (RPE) were significantly higher in the patients compared to control subjects at any absolute workload during the cycle test (Fig 3.13). However, control values were significantly higher at 50 and 75 % VO_2 peak ($p < 0.05$; Fig 3.14).

Exercise performance and cardiovascular and respiratory measurements at the peak exercise workload

All patients and control subjects completed the maximal exercise tests to exhaustion. Cardiorespiratory responses and perception of effort at the peak workload are listed in Table 3.5.

Peak workload and exercise time completed by the patient group was significantly lower compared to the control group ($p < 0.01$). Mean peak HR and mean peak SBP were also significantly lower in the patients compared to the control subjects ($p < 0.01$; $p < 0.05$ respectively). $\dot{\text{V}}\text{O}_2$ peak and maximum $\dot{\text{V}}\text{i}$ were significantly lower in the patients compared to control subjects at peak workload ($p < 0.01$), as was the respiratory exchange ratio (RER; $p < 0.05$). Peak blood lactate concentrations were significantly lower in the patient group compared to controls ($p < 0.01$).

Seven patients terminated exercise due to leg muscle fatigue, two patients complained of fatigue of the arm muscles from holding onto the bicycle handle-bars, that is, this was not ischaemic pain, and two patients complained of general fatigue. Eight control subjects terminated exercise complaining of general fatigue, whilst two subjects complained of leg fatigue.

Table 3.5. Cardiorespiratory measurements and performance variables at the peak exercise workload

| Patient | Peak WL (Watts) | Exercise time (min) | HR (b/min) | SBP (mmHg) | VO ₂ peak (mlO ₂ /kg/min) | V _i (L/min) | RER (VCO ₂ /VO ₂) | Plasma Lactate (mmol/l) |
|----------------|-----------------|---------------------|--------------|-------------|---|------------------------|--|-------------------------|
| 1 | 30 | 6 | 124 | 110 | 8.5 | 31.8 | 1.06 | 4.1 |
| 2 | 50 | 8 | 141 | 115 | 11.4 | 37.3 | 1.01 | 4.9 |
| 3 | 80 | 11 | 162 | 148 | 17.8 | 28.9 | 1.01 | 5.2 |
| 4 | 60 | 9 | 145 | 110 | 11.1 | 33.1 | 1.02 | 5.2 |
| 5 | 80 | 11 | 130 | 110 | 12.9 | 24.7 | 0.95 | 6.4 |
| 6 | 50 | 8 | 137 | 85 | 10.6 | 34.9 | 0.97 | 3.3 |
| 7 | 50 | 8 | 106 | 90 | 8.4 | 32.8 | 0.98 | 2.7 |
| 8 | 100 | 13 | 134 | 200 | 13.8 | 39.8 | 1.06 | 3.9 |
| 9 | 40 | 7 | 120 | 110 | 10.1 | 28.8 | 0.86 | 3.1 |
| 10 | 140 | 16 | 182 | 210 | 17.6 | 54.5 | 1.11 | 8.1 |
| 11 | 120 | 15 | 138 | 210 | 14.9 | 50.8 | 1.03 | 3.9 |
| Mean | 73** | 10** | 138** | 136* | 12.5** | 36.1** | 1.01* | 4.6** |
| SEM | 10 | 1 | 6 | 14 | 1.0 | 2.7 | 0.01 | 0.5 |
| Control | | | | | | | | |
| 1 | 170 | 20 | 157 | 230 | 20.0 | 59.0 | 1.05 | 6.8 |
| 2 | 275 | 25 | 188 | 160 | 51.0 | 103.2 | 1.00 | 7.8 |
| 3 | 265 | 24 | 159 | 190 | 39.0 | 83.8 | 1.10 | 6.7 |
| 4 | 200 | 16 | 200 | 200 | - | - | - | - |
| 5 | 275 | 25 | 180 | 180 | 37.0 | 71.2 | 1.20 | 8.2 |
| 6 | 195 | 17 | 195 | 195 | - | - | - | 7.8 |
| 7 | 230 | 26 | 143 | 190 | 33.8 | 82.4 | 1.10 | 7.6 |
| 8 | 150 | 18 | 172 | 170 | 27.1 | 67.0 | 1.20 | 7.8 |
| 9 | 240 | 27 | 180 | 210 | 26.6 | 72.7 | 1.18 | 5.9 |
| 10 | 240 | 27 | 176 | 220 | 39.7 | 90.2 | 1.12 | 7.2 |
| Mean | 224 | 23 | 175 | 194 | 34.3 | 78.7 | 1.12 | 7.3 |
| SEM | 14 | 1 | 6 | 7 | 3.5 | 5.0 | 0.03 | 0.3 |

Abbreviations: WL, workload; VO₂ peak, peak oxygen consumption; SBP, systolic blood pressure; HR, heart rate; b/min, beats per minute; mlO₂/kg/min, millilitres of oxygen consumed per kilogram of body weight per minute of exercise; V_i, ventilation; l/min, litres per minute; RER, respiratory exchange ratio; VCO₂, volume of carbon dioxide; mmol/l, millimole per litre; SEM, standard error of the mean. Values are expressed as the mean ± SEM. * = p<0.05 ** = p<0.01 patient vs control.

Skeletal muscle function

Parameters of isokinetic and isometric skeletal muscle function are listed in Table 3.6. Isokinetic peak torques of the quadriceps and hamstring muscle groups in patients were significantly lower than in controls (p<0.05). However, when corrected for lean thigh volume, values were not significantly different from controls. Total work performed during 25 contractions by the quadriceps and hamstring muscle groups was also significantly lower in the patients (p<0.05). But when corrected for lean thigh volume, total work of the quadriceps and hamstring muscles was still significantly lower than in the control subjects (p<0.05).

Isometric maximum voluntary contraction (MVC) generated by the quadriceps muscle group was significantly lower in patients than in controls ($p < 0.001$). However, when corrected for LTV, the MVC was no longer statistically different between groups.

Time to fatigue measured during repeated maximal isometric relaxation/ contraction cycles was also significantly lower in patients compared to controls ($p < 0.05$).

Table 3.6. Isometric and isokinetic tests of skeletal muscle function

| Patient | PKTQ (Nm) | PKTQ/ LTV (Nm/cc) | PKTH (Nm) | PKTH/ LTV (Nm/cc) | TWQ (J) | TWQ / LTV (J/cc) | TWH (J) | TWH/ LTV (J/cc) | MVC (Nm) | MVC / LTV (Nm/cc) | TTF (sec) |
|----------------|--------------|-------------------------|--------------|-------------------------|--------------|------------------------|-------------|-----------------------|--------------|-------------------------|--------------|
| 1 | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 94 | 30 | 51 | 16 | 1161 | 366 | 448 | 142 | 110 | 35 | 30 |
| 3 | 120 | 32 | 48 | 13 | 1222 | 331 | 298 | 93 | 150 | 40 | 91 |
| 4 | 135 | 58 | 37 | 16 | 2075 | 894 | 480 | 206 | 110 | 47 | 62 |
| 5 | 121 | 52 | 67 | 29 | 1541 | 664 | 448 | 195 | 110 | 48 | 30 |
| 6 | 133 | 33 | 36 | 9 | 1500 | 376 | 976 | 245 | 120 | 30 | 68 |
| 7 | 105 | 25 | 54 | 15 | 1175 | 289 | 124 | 31 | 120 | 30 | 59 |
| 8 | 196 | 56 | 85 | 27 | 2223 | 635 | 458 | 131 | 120 | 34 | 59 |
| 9 | 105 | 33.9 | 40 | 13 | 900 | 257 | 111 | 36 | - | - | - |
| 10 | 192 | 66.4 | 90 | 26 | 2380 | 823 | 1300 | 371 | 190 | 66 | 75 |
| 11 | 124 | 35.4 | 85 | 29 | 1467 | 419 | 1396 | 483 | 180 | 51 | 139 |
| Mean | 133* | 42 | 60 * | 19 | 1565* | 506* | 604* | 192* | 134** | 42 | 68 * |
| SEM | 15 | 6 | 8 | 2 | 166 | 70 | 163 | 41 | 12 | 5 | 12 |
| Control | | | | | | | | | | | |
| 1 | 168 | 40 | 78 | 19 | 2641 | 633 | 1570 | 376 | 230 | 55 | 116 |
| 2 | 291 | 69 | 169 | 40 | 4662 | 1099 | 3619 | 853 | 190 | 45 | 80 |
| 3 | 292 | 54 | 169 | 31 | 3998 | 737 | 3018 | 556 | 170 | 31 | 160 |
| 4 | 230 | 48 | 149 | 31 | 3185 | 664 | 2749 | 573 | 230 | 48 | 75 |
| 5 | 225 | 47 | 131 | 28 | 3615 | 759 | 2414 | 507 | 150 | 32 | 150 |
| 6 | 292 | 61 | 150 | 32 | 3467 | 729 | 2617 | 550 | 145 | 31 | 70 |
| 7 | 112 | 28 | 66 | 16 | 2300 | 566 | 1619 | 398 | 168 | 41 | 92 |
| 8 | 102 | 37 | 33 | 12 | 1241 | 446 | 431 | 155 | 200 | 72 | 75 |
| 9 | 156 | 41 | 65 | 17 | 2302 | 603 | 913 | 239 | 210 | 55 | 80 |
| 10 | 163 | 49 | 68 | 20 | 1504 | 501 | 1084 | 324 | 248 | 75 | 97 |
| Mean | 203 | 47 | 108 | 25 | 2892 | 674 | 2003 | 453 | 194 | 49 | 100 |
| SEM | 23 | 4 | 16 | 3 | 345 | 59 | 326 | 63 | 11 | 5 | 10 |

Abbreviations: PKTQ, peak torque produced by the quadriceps muscles; PKTH, peak torque produced by the hamstring muscles; Nm, Newton-metres; LTV, lean thigh volume; cc, cubic centimetres; TWQ, total work performed by the quadriceps muscles; TWH, total work performed by the hamstring muscles; MVC, maximum voluntary contraction, TTF, time to fatigue; SEM, standard error of the mean. * = $p < 0.05$ ** = $p < 0.001$ patient vs control.

Discussion

The first important finding of this study was that exercise tolerance was significantly reduced in patients with CHF and appeared to be limited by peripheral (skeletal muscle) rather than by central factors. Evidence for this conclusion was found during the incremental cycle test to exhaustion and during isokinetic testing of skeletal muscle function. This conclusion conflicts with the more traditional view that central cardiovascular factors may limit the exercise capacity of patients with CHF (Packer 1990; Wilson et al. 1984b; Weber et al. 1982).

Thus patients with CHF terminated progressive cycle exercise to exhaustion at heart rates, rates of ventilation, respiratory exchange ratios and, most especially, blood lactate concentrations that were significantly lower than values achieved by control subjects during maximal dynamic exercise (*Table 3.5*). Furthermore, rates of O₂ consumption did not plateau during graded maximal exercise. If central cardiovascular function limited their exercise performance, one would have expected a plateau of O₂ consumption and values for all these measurements to be higher in patients with CHF than in the control subjects. Indeed, if skeletal muscle blood flow was impaired as a result of CHF, according to the proposal that anaerobic conditions in muscle favour skeletal muscle lactate production, one might have expected blood lactate concentrations to be very high at fatigue in patients with CHF. But this was not found. Other research in this laboratory (Kempeneers et al. 1990; Diesel et al. 1990) has previously used this line of reasoning to argue that patients with chronic renal disease also have a myopathy that limits their exercise tolerance (Diesel et al. 1993). Kavanagh and Yacoub (1992) reported a similar finding in cardiac transplant patients and have also concluded that the exercise tolerance of these patients is limited by peripheral factors.

More direct evidence for a peripheral, skeletal muscular limitation was the finding on isokinetic muscle function testing. Performance in these tests is not considered to be limited by central cardiovascular function as only a small muscle mass is active during exercise. We found that isokinetic peak torque and the MVC of the quadriceps muscles was significantly reduced in patients compared to controls (*Table 3.6*). But when corrected for lean thigh volume, MVC and peak torques were no longer significantly different from control values, as also found by Minotti et al. (1991b).

This finding is therefore similar to others which have demonstrated that maximal force production per unit muscle cross-sectional area is probably within the normal range in patients with CHF (Magnusson et al. 1994; Buller et al. 1991) but that muscle mass is reduced, thereby reducing the total force production by muscle in such patients. However Poole-Wilson et al. (1988) reported that

even when corrected for the (reduced) lean thigh volume, isokinetic skeletal muscle function was still impaired in cardiac patients.

Although force production measured during maximum contractions of short duration was not different to control subjects, the total work performed during repeated maximal isokinetic contractions was significantly lower in patients than in control subjects. These differences persisted even when measurements were corrected for differences in lean thigh volume. This indicates that as the tests of muscle function become prolonged and include an endurance component, the muscle function of these patients becomes progressively more abnormal. This would indicate that, whilst the skeletal muscles of patients with CHF retain the ability to produce maximum force, they fatigue more rapidly and therefore produce less force during subsequent consecutive contractions. As a result, the total work performed during a more prolonged test of skeletal muscle function, is reduced. This finding was confirmed by the test of repetitive isometric contraction/relaxation cycles in which time to fatigue was significantly shorter in patients with CHF (*Table 3.6*).

Thus our results indicate that the resistance to the onset of fatigue (Coetzer et al. 1993; Derman et al. 1993) is altered in these patients. It is possible that a smaller percentage of Type I fibres in patients with CHF (Mancini et al. 1989; Sullivan et al. 1990a) which would be expected to cause a more rapid rate of phosphocreatine depletion during exercise (Mancini et al. 1992; Rajagopalan et al. 1988) may explain this finding.

We also found that the lean thigh volume of patients was significantly less than in controls. Anorexia, physical inactivity, increased catabolic state, and increases in the serum concentrations of cortisol, ACTH and tumor necrosis factor could possibly all contribute to muscle atrophy in patients with CHF (Mancini et al. 1992; Peterson et al. 1988; Levine et al. 1990; Francis et al. 1990). It is unlikely that inactivity alone contributed to the reduction in skeletal muscle mass since the control subjects were also inactive.

It has been suggested that the reduced skeletal muscle mass alone could explain the exercise intolerance of these patients (Martin et al. 1989; Jondeau et al. 1992; Mancini et al. 1988; Massie et al. 1987a). However, Mancini et al. (1992) did not find a significant correlation between VO_2 peak and lean body mass, weight, or triceps skinfold thickness and concluded that skeletal muscle atrophy contributes modestly to both the reduced exercise capacity and the altered skeletal muscle metabolism of patients with CHF. Our finding that one fundamental characteristic of skeletal muscle function, namely fatigue resistance, is altered in CHF, is compatible with this conclusion.

The second important finding of this study is that submaximal O_2 consumption and minute ventilation are not different in patients with CHF compared with controls at low work rates. Indeed our data suggest that O_2 consumption, minute ventilation and blood lactate concentrations are appropriate for low workloads in these patients. But when considering these variables at exercise intensities relative to percentage VO_2 max, O_2 consumption, minute ventilation and blood lactate concentrations are decreased in patients with CHF compared to controls. These findings are probably due to the greater absolute maximum workload achieved by the controls or, stated differently, indicate that the maximal workloads achieved by the patients in heart failure are abnormally low.

It has been suggested that poor exercise tolerance and increased perception of effort might be explained by early onset of anaerobic metabolism and metabolic acidosis (Koike et al. 1990). Many recent studies have noted a close association between early metabolic acidosis and abnormal exercise capacity in patients with CHF (Weber and Janicki 1985; Roubin et al. 1990; Weber et al. 1982; Wilson and Ferraro 1983; Wilson et al. 1984). Our data indicate that at identical work rates (0-100W), blood lactate concentrations, rates of O_2 consumption and minute ventilation were not different between patients and controls (Figs 3.5-3.12). The equivalent blood lactate concentrations during submaximal exercise in patients with chronic heart failure and controls provide no evidence for early anaerobiosis during exercise in the patients with CHF. Rather, our findings demonstrate a gradual and appropriate increase in blood lactate concentrations during graded exercise in these patients. Similar findings were described by Simonton et al. (1988).

However, Sullivan et al. (1989) demonstrated that systemic venous lactate concentrations and the rate of lactate production by the active skeletal muscles was increased in patients with CHF compared to control subjects at low workloads, while whole body VO_2 (ml/min) values were not different between groups at those low workloads. But the VO_2 of the active muscle mass was less in patients than in controls. The authors conclude that early anaerobiosis causing increased lactate production and accumulation does indeed occur in patients with CHF.

These findings are at variance with the findings in the present study which show no evidence of altered rates of oxygen consumption and systemic venous lactate accumulation in patients with CHF compared with controls. This might be due to the different exercise protocols and patient characteristics from the above mentioned study. However it is possible that differences in blood lactate production and O_2 consumption might only be evident when measured directly over the active skeletal muscle in the femoral arterial and venous blood. It is further possible that these

changes are not mirrored by changes in systemic venous lactate concentrations, perhaps due to increased clearance of lactate by the liver. This would only be resolved by using the same invasive techniques as those used by Sullivan et al. (1989).

Sullivan et al. (1989) did not report ratings of perceived exertion during exercise. The present study shows that ratings of perceived exertion at identical work rates are significantly higher in patients with CHF compared to controls, while blood lactate concentration, O_2 consumption and minute ventilation are the same. This finding indicates that factors other than increased blood lactate concentrations, O_2 consumption and minute ventilation are responsible for increased perception of effort during graded exercise in patients with CHF.

In summary, patients with CHF have altered skeletal muscle function, including a decreased resistance to the onset of fatigue. Furthermore, graded exercise to exhaustion in these patients seems to be limited more by peripheral rather than central factors. It is possible that skeletal muscle structural abnormalities might be related to these abnormalities of skeletal muscle function. This possibility will be addressed in Chapter 4.

CHAPTER 4

STRUCTURAL ABNORMALITIES OF SKELETAL MUSCLE IN PATIENTS WITH CHRONIC HEART FAILURE: RELATIONSHIP TO EXERCISE PERFORMANCE

Introduction

As described in Chapter 1, studies have documented gross changes in the histology of the skeletal muscles in patients with chronic heart failure (CHF) (Lipkin et al. 1988; Drexler et al. 1992), but few studies have provided a detailed description of these changes, and none have provided equivalent information from appropriate control groups. Furthermore, few studies have examined the ultrastructure of the skeletal muscle in patients with CHF.

Minotti et al. (1991a) and others have shown that the extent of metabolic abnormalities in the skeletal muscle correlate with the extent of exercise intolerance in these patients and could contribute to their impaired exercise tolerance (Wiener et al. 1986; Massie et al. 1987a, b; Sullivan et al. 1990, 1991; Drexler et al. 1988, 1992; Mancini et al. 1992; 1994). Furthermore, Drexler et al. (1992) reported a relationship between mitochondrial volume density and VO_2 peak in patients with CHF.

But it is not known if the severity of the histological abnormalities of the skeletal muscles in patients with CHF relate to the exercise intolerance experienced by these patients. Only crude methods for quantification of skeletal muscle structural damage exist which do not include all histological features of the skeletal myopathy (Sapega et al. 1985). We therefore sought to create a method for quantification which would include all aspects of skeletal muscle structural damage.

Accordingly, the purpose of this study was (i) to describe the histological and ultrastructural findings in skeletal muscle biopsy samples from patients with CHF; (ii) to develop a simple histological grading system to quantify the magnitude of these changes, thereby allowing the prospective assessment of changes due to drugs, exercise training or disease progression; and (iii) to determine the relationship of these changes to alterations in the exercise capacity of these patients.

Skeletal muscle biopsy procedures

Skeletal muscle biopsy of the vastus lateralis was performed according to the methods described in Chapter 2. A skeletal muscle biopsy was only performed if the clotting factor (INR; International Ratio) was < 2.0 . Ten of the patients with CHF studied in Chapter 3 consented to a skeletal muscle biopsy, whilst a skeletal muscle biopsy could not be performed on one patient who had an INR > 2.0 . Eight control subjects consented to a skeletal muscle biopsy.

Histological scoring of skeletal muscle biopsy samples

The skeletal muscle biopsy samples for light microscopic analysis from both patients and controls subjects were numbered, randomized and examined by two pathologists who had no knowledge of the origin of the biopsies. Each specimen was closely examined for the presence of the characteristics of the skeletal myopathy associated with CHF, namely: fibre atrophy, fibre hypertrophy, fibre necrosis, fibre grouping, fibre splitting, presence of moth-eaten fibres, fibrosis, nuclear abnormalities including presence of nuclear chains, knots, vesicular and internal nuclei, phagocytosis, lipid inclusions, basophilia and capillary thickening. Each identified abnormality was graded according to the following system; 1 = mild, 2 = moderate, 3 = severe. Skeletal muscle pathology scores were then summed for each specimen and the mean score of the pathologists was taken as the pathology score for that patient or control subject.

Results

Morphometry of skeletal muscle fibre type, size, distribution and predominance is described in Table 4.1

Skeletal muscle fibre morphometry

Measurement of the skeletal muscle fibres stained for myosin ATPase, from the patient group revealed a mean Type I fibre size of $67 \pm 3 \mu\text{m}$ and a mean Type II fibre size of 60 ± 4 micrometres (μm). One patient had significant Type II fibre atrophy (mean diameter = $40 \mu\text{m}$; Dubowitz et al. 1985) and one patient had significant hypertrophy of both Type I and II muscle fibres. The mean percentage of Type II skeletal muscle fibres in the patient group was $65 \pm 4 \%$. Two patients displayed Type II fibre predominance ($>80\%$). Seventeen $\pm 6\%$ of Type II fibres were atrophic, whilst $17 \pm 7\%$ of Type II fibres were hypertrophic. Only $2 \pm 1\%$ of Type I fibres showed significant atrophy whilst $10 \pm 2\%$ of Type I fibres were hypertrophied.

Morphometric analysis of control biopsies revealed a mean Type I fibre size of $70 \pm 9 \mu\text{m}$ and a mean Type II fibre size of $70 \pm 9 \mu\text{m}$. One subject had significant hypertrophy of Type II muscle fibres. The mean percentage of Type II skeletal muscle fibres was $56 \pm 4 \%$.

Table 4.1 Morphometry of skeletal muscle fibre type, size, distribution and predominance

| Patient | Fibre Size (micrometres) | | Fibre Distribution | | | Predominance | |
|----------------|--------------------------|-----------|--------------------|------------|--------------|--------------|-----------|
| | Type I | Type II | Type I | Type II | Total fibres | Type I% | Type II% |
| 1 | 66 | 66 | 48 | 104 | 152 | 32 | 68 |
| 2 | 53 | 41 | 80 | 102 | 182 | 44 | 56 |
| 3 | 90 | 81 | 64 | 110 | 174 | 38 | 63 |
| 4 | 67 | 56 | 41 | 106 | 147 | 28 | 72 |
| 5 | 58 | 49 | 48 | 100 | 148 | 32 | 68 |
| 6 | 61 | 66 | 62 | 100 | 162 | 38 | 62 |
| 7 | 66 | 55 | 112 | 80 | 192 | 58 | 42 |
| 8 | 68 | 40 | 32 | 141 | 173 | 18 | 82 |
| 10 | 70 | 78 | 19 | 106 | 125 | 15 | 85 |
| 11 | 72 | 67 | 79 | 99 | 178 | 44 | 56 |
| Mean | 67 | 60 | 59 | 105 | 163 | 35 | 65 |
| SEM | 3 | 4 | 8 | 5 | 6 | 4 | 4 |
| Control | | | | | | | |
| 1 | 81 | 75 | 46 | 92 | 138 | 33 | 67 |
| 2 | 73 | 70 | 83 | 117 | 200 | 42 | 56 |
| 3 | 63 | 78 | 115 | 85 | 200 | 58 | 42 |
| 4 | 74 | 77 | 71 | 100 | 171 | 42 | 58 |
| 7 | 63 | 47 | 55 | 103 | 158 | 35 | 65 |
| 8 | 73 | 80 | 51 | 69 | 120 | 43 | 58 |
| 9 | 60 | 65 | 103 | 82 | 185 | 56 | 44 |
| 10 | 68 | 66 | 72 | 99 | 180 | 48 | 50 |
| Mean | 70 | 70 | 75 | 93 | 168 | 44 | 56 |
| SEM | 3 | 3 | 10 | 12 | 12 | 4 | 4 |

Light Microscopy

Details of the light microscopic findings of individual biopsies are presented in *Table 4.2*. Normal size and distribution of skeletal muscle fibres from a control subject is depicted in *Figure 4.1*. Atrophic and hypertrophic fibres were present in all patients and most also had fibres showing splitting and central nuclei (*Figure 4.2*). The atrophied and hypertrophied fibres were usually both Types I and II but Type II fibres were more severely atrophic. The fibres of two patients showed marked hypertrophy.

Nuclear chains or nuclear clumps, some pyknotic, were prominent. Occasional fibres appeared necrotic and phagocytes were present. Atrophic fibres showed disturbed patterns of NADH

staining and occasional fibres had architectural changes. Degenerative features were uncommon (Figure 4.3).

Some patients appeared to have more lipid droplets within the fibres than did control subjects. Thickening of the capillary basement membrane was visible on light microscopy in all but one patient. Slits or cracks in fibres containing NADH positive material were also seen in a number of patients.

One control biopsy displayed scanty necrotic fibres, fibre splitting and some moth-eaten fibres, and another showed a mild increase in lipid and thickening of the capillary basement membranes. The extent and severity of these changes were not as pronounced as the abnormalities present in the biopsies from the patient group.

Electron Microscopy

The electron microscopic findings are presented in *Table 4.3*. The ultrastructural changes include the presence of nuclear chains with folding of the nuclear membranes and nuclear clumps. In some patients, clumping was associated with chromatin margination. Dissolution of the myofilaments and Z-bands was present in 7 patients. Glycogen aggregation was both subsarcolemmal and intermyofibrillar (Figure 4.4). Examination of the mitochondria revealed a spectrum of changes comprising swelling, disruption and a loss of cristae, abnormal cristae formation and whorled inclusions (Figures 4.4 & 4.5). Lipid accumulation was mainly in the subsarcolemmal region.

Some biopsies contained abundant lipofuscin. Subsarcolemmal filamentous bodies consisting of whorled microfilaments were present in three patients (Figure 4.6); these were associated with sarcolemmal folding in two cases and redundant sarcoplasmic basement membranes in one case (Figure 4.7). Two others revealed thickening of the sarcoplasmic basement membranes while five showed increased collagen in the interstitium (Figure 4.7). Macrophages and mast cells were also frequently present in the interstitium.

The capillary basement membranes of all patients were strikingly thickened. Reduplication, entrapped debrinous material together with swollen lucent capillary endothelium were also seen (Figure 4.8). Ultrastructural morphometry revealed capillary basement membrane width varying from 194 to 840 nm (mean \pm SEM width 409 ± 13 nm).

Table 4.2 Light microscopic analysis of individual skeletal muscle biopsy samples

| Patient | Light Microscopy | Pathology score (arbitrary units) |
|----------------|---|--|
| 1 | Mild fibre atrophy (moth eaten on NADH), moderate hypertrophy, fibre splitting, internal nuclei, nuclear chains and pyknotic knots. Occasional necrotic fibres with phagocytes and occasional fibres with architectural changes. Marked capillary basement membrane (CBM) thickening. | 16 |
| 2 | Marked atrophy with mild hypertrophy, fibre splitting, pyknotic nuclear knots. Basophilic fibres, fibre size variation, occasional phagocytes. Some adipose infiltration. CBM thickening. | 13 |
| 3 | Occasional atrophic or split fibres, prominent hypertrophied fibres, moth eaten fibres (NADH stain), central nuclei. Moderate numbers of nuclear chains and pyknotic clumps. Occasional architectural changes. CBM thickening slight. | 8 |
| 4 | Occasional atrophic hypertrophic or regenerating fibres. Some nuclear chains and pyknotic knots. Occasional necrotic cells with phagocytes. Marked CBM thickening. | 8 |
| 5 | Extensive atrophy (with I and II), moderate fibre splitting, occasional moth eaten fibres (NADH) and pyknotic nuclear clumps. Occasional necrotic fibres with phagocytes. Moderate CBM thickening. | 11 |
| 6 | Mild atrophy (chiefly Type II), some hypertrophy, moderate fibre splitting, internal nuclei, nuclear chains and pyknotic clumps. Occasional necrotic cells. Moth eaten fibres (NADH). Mild CBM thickening. | 17 |
| 7 | Fibre size variation, mild atrophy and hypertrophy, moderate fibre splitting, internal nuclei, nuclear chains and pyknotic knots. Occasional necrotic cells with phagocytes. Moderate CBM thickening. | 12 |
| 9 | Marked atrophy with some hypertrophy, moderate numbers of necrotic cells with phagocytosis, marked fibre splitting, internal nuclei, nuclear chains and pyknotic clumps. Moth eaten fibres (NADH) and some architectural changes. Marked CBM thickening. | 26 |
| 10 | Mild atrophy, marked hypertrophy, prominent internal nuclei and necrotic fibres with phagocytosis, some pyknotic nuclear clumps. | 6 |
| 11 | Moderate atrophy and hypertrophy, scattered necrotic fibres with phagocytes, occasional fibre splitting and pyknotic nuclear clumps. Mild CBM thickening. | 6 |
| Control | | |
| 1 | Normal skeletal muscle. | 0 |
| 2 | Normal skeletal muscle | 0 |
| 3 | Normal skeletal muscle, few split fibres. | 1 |
| 4 | Normal skeletal muscle | 0 |
| 7 | Mild increase in internal nuclei and lipid, early ragged red fibers, arterioles thickened. | 3 |
| 8 | Some necrotic fibers, some of which are moth-eaten on NADH stains, and a small amount of splitting is present. (This patient was diagnosed with cancer of the stomach eight months after this biopsy was performed). | 5 |
| 9 | Normal skeletal muscle. | 0 |
| 10 | Normal skeletal muscle. | 0 |

Table 4.3. Electron microscopic analysis of individual skeletal muscle biopsy samples

| Patient | Electron Microscopy |
|----------------|--|
| 1 | Nuclear chains, Subsarcolemmal lipid accumulation, Intermyoibrillar glycogen aggregation macrophages, Numerous lipofuscin granules, Degenerate muscle cells, Capillary basement membrane thickening. |
| 2 | Z-band streaming, Redundant sarcoplasmic basement membranes, Increased interstitial collagen and glycoimmunoglycans, Macrophages, Mast cells, Thickening and multilayering of the capillary basement membrane which contain entrapped debrinous material. |
| 3 | Loss of myofilaments and Z-banding, Increased lipid accumulation and intermyofibrillar glycogen aggregates, Mitochondrial inclusions, Filamentous bodies, Numerous subsarcolemmal myelin inclusions, Sarcolemmal folding, Macrophages, Capillary basement membrane thickening with mild multilayering. |
| 4 | Nuclear pyknosis and cellular degeneration, Myofilament and Z-band dissolution, Large mitochondrial myelin inclusions, Subsarcolemmal vacuolation, Filamentous bodies, Sarcolemmal folding, Macrophages containing haemosiderin pigment, Increased interstitial collagen, Capillary basement membrane thickening with entrapped granular debris. |
| 5 | Increased lipid accumulation, Subsarcolemmal glycogen aggregation, Mast cells, Thickened capillary basement membranes, Swollen capillary endothelium. |
| 6 | Nuclear chains, Myofilament and Z-band loss, Small mitochondrial inclusions, Subsarcolemmal lipid accumulation and vacuolation, Numerous lipofuscin pigment, Thickening of the sarcolemmal basement membranes, Redundant sarcoplasmic basement membranes, Increased interstitial collagen and glycosaminoglycans, Myoblasts, Macrophages, Capillary basement membrane thickening with multilayering and entrapped granular material. |
| 7 | Extensive myofilamentous and Z-band dissolution, Mitochondrial myelin inclusions, Lipid accumulation, Glycogen aggregates, Sarcolemmal folding, Increased interstitial collagen, Capillary basement membrane thickening and multilayering. |
| 9 | Margination of the nuclear chromatin, A loss of myofilaments and Z-banding, Whorled mitochondrial inclusions, Numerous lipofuscin granules, Sarcolemmal folding and thickening of the sarcolemmal basement membranes, Increased interstitial collagen, Macrophages, Severe thickening of the capillary basement membranes with entrapped debrinous material and collagen fibres, Swollen capillary endothelial cells. |
| 10 | Loss of myofilaments and Z-banding, Splitting of the myofibrils, Numerous pleomorphic mitochondria, Lipid accumulation, Increased interstitial collagen and glycosaminoglycans, Thickened capillary basement membranes. |
| 11 | Loss of myofilaments and Z-banding, Lipid accumulation, Subsarcolemmal glycogen aggregates, Filamentous bodies, Degenerate cells, Macrophages, Mast cells, Capillary basement membrane thickening and swollen endothelium. |
| Control | |
| 1 | Normal skeletal muscle with increased lipid. |
| 2 | Normal skeletal muscle |
| 3 | Normal skeletal muscle |
| 4 | Normal skeletal muscle with mast cells and a single cell with increased subsarcolemmal glycogen aggregation |
| 7 | Normal skeletal muscle. |
| 8 | Normal with some disrupted mitochondria. |
| 9 | Normal skeletal muscle. |
| 10 | Normal with some disrupted mitochondria. |

Evidence for muscle regeneration was present. Regenerated myocytes showed multiple nuclei, marginated chromatin, prominent nucleoli, randomly arrayed actin and myosin filaments and rudimentary Z-banding (Figure 4.9). Degenerate muscle cells with clumped chromatin, prominent nucleoli, disrupted cytoplasmic myofibrils, vacuolation, distended mitochondria (M) and lipofuscin were also present (Figure 4.10).

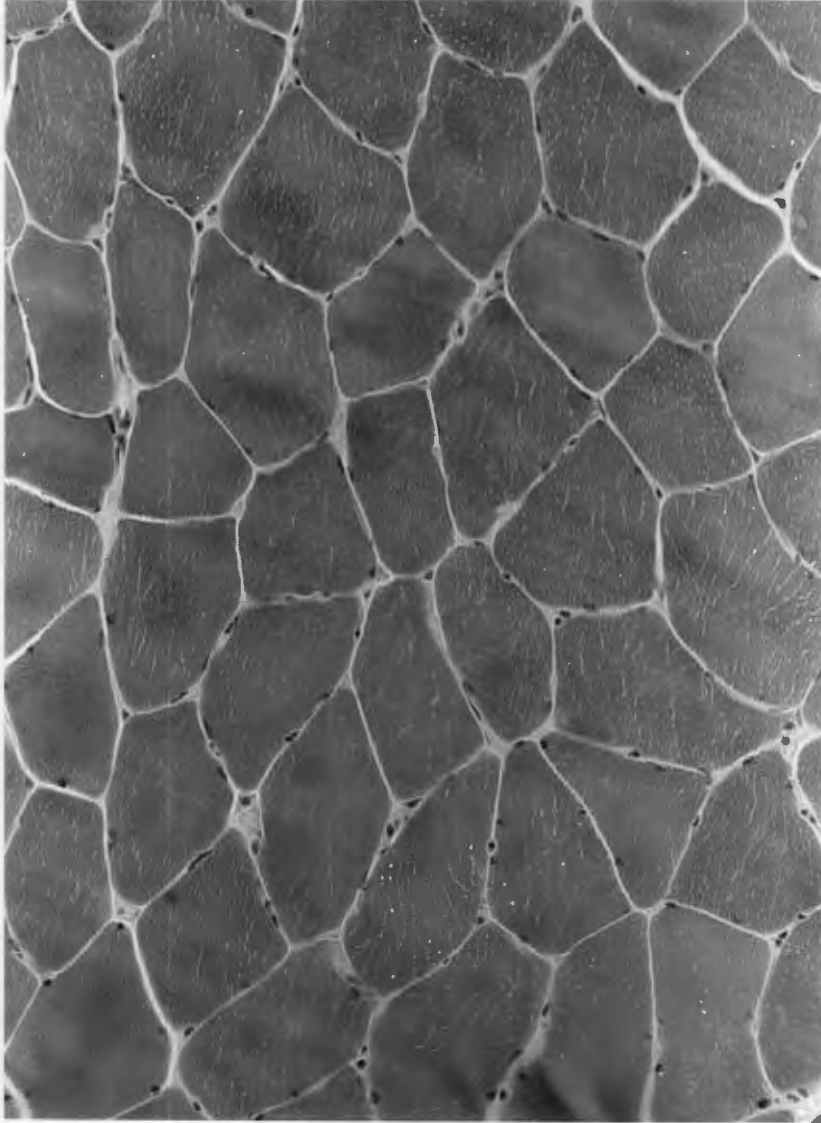


Fig 4.1. A light photomicrograph showing normal skeletal muscle from a control subject (H&E x 200).



Fig 4.2. A light photomicrograph showing prominent atrophy, hypertrophy and split fibres. There are occasional central nuclei and capillaries with very thick walls can be seen.

(H&E x 200)

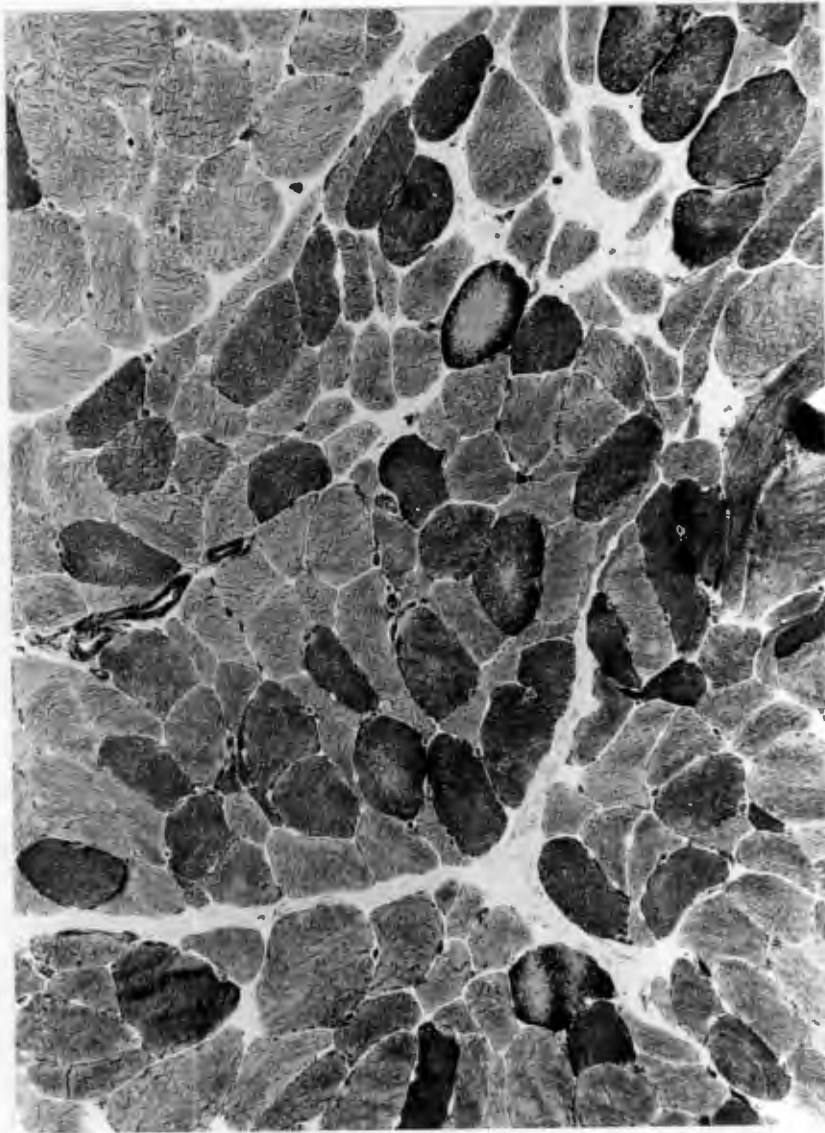


Fig 4.3. A NADH stain showing moth-eaten Type I fibers. Note that the atrophy and hypertrophy affects both skeletal muscle fiber types. (NADH x 120).

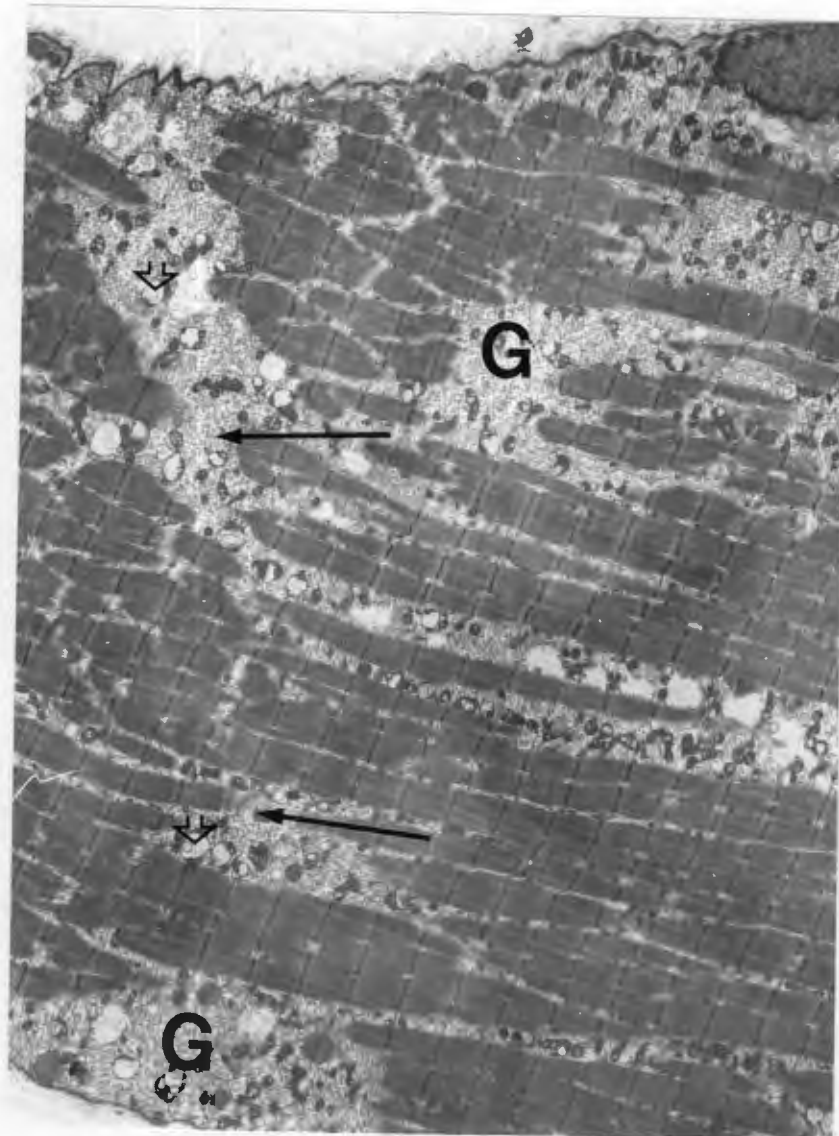


Fig 4.4. Electron micrograph showing a loss of myofibrils (arrows), prominent subsarcolemmal and intermyofibrillar glycogen (G) and swollen mitochondria with lost cristae (arrow heads). (x 5500)

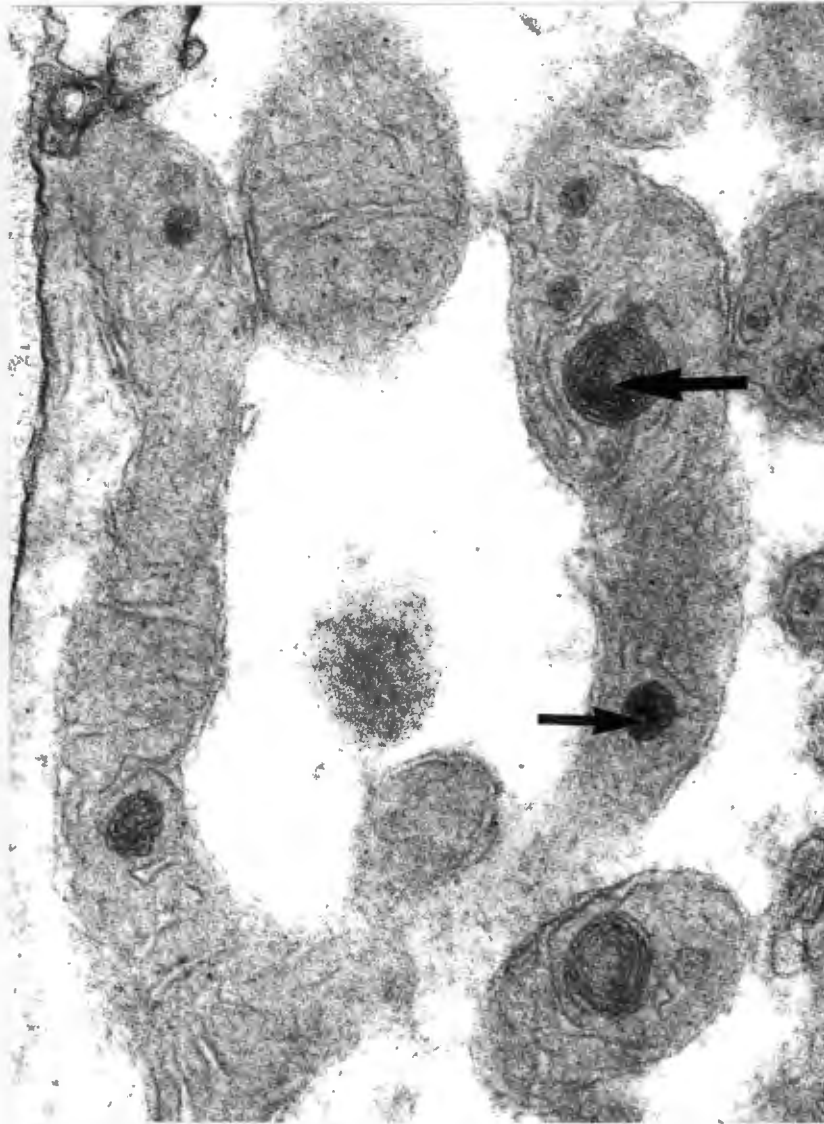


Fig 4.5. Electron micrograph of myelin whorl formation within the mitochondria (arrows). (x 72000)

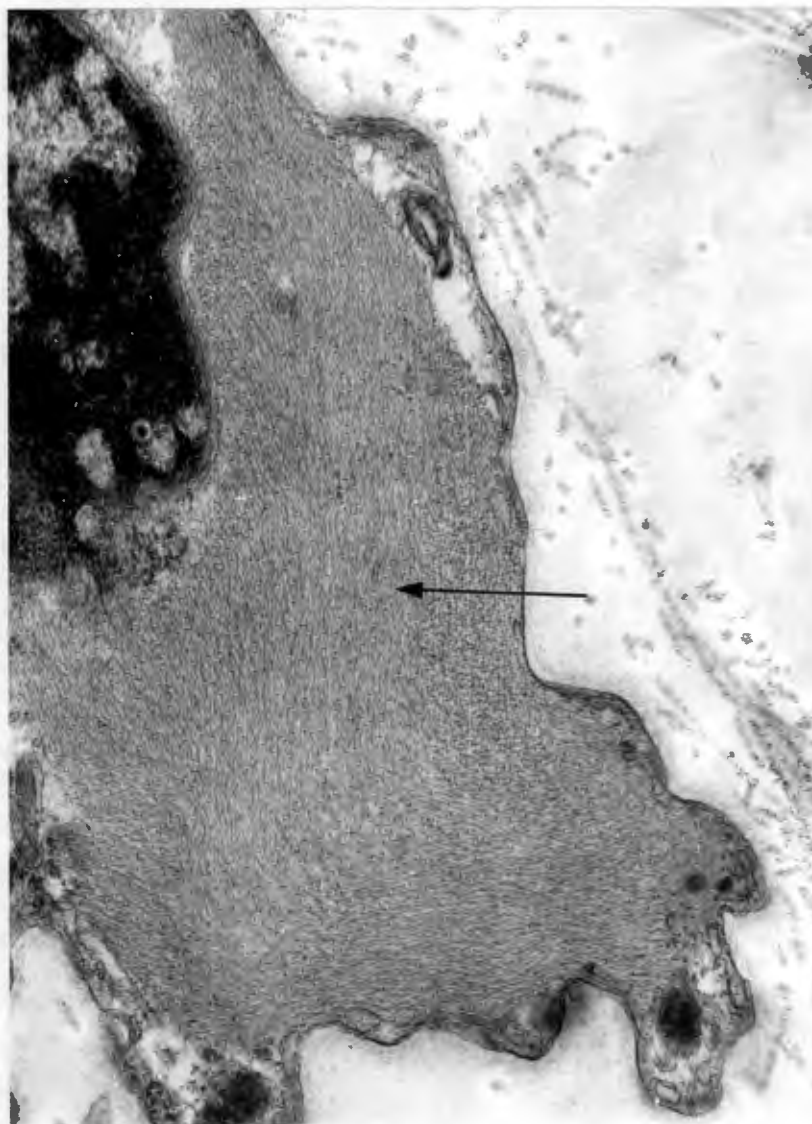


Fig 4.6. Electron micrograph of a subsarcolemmal filamentous body (arrow). (x 44000)

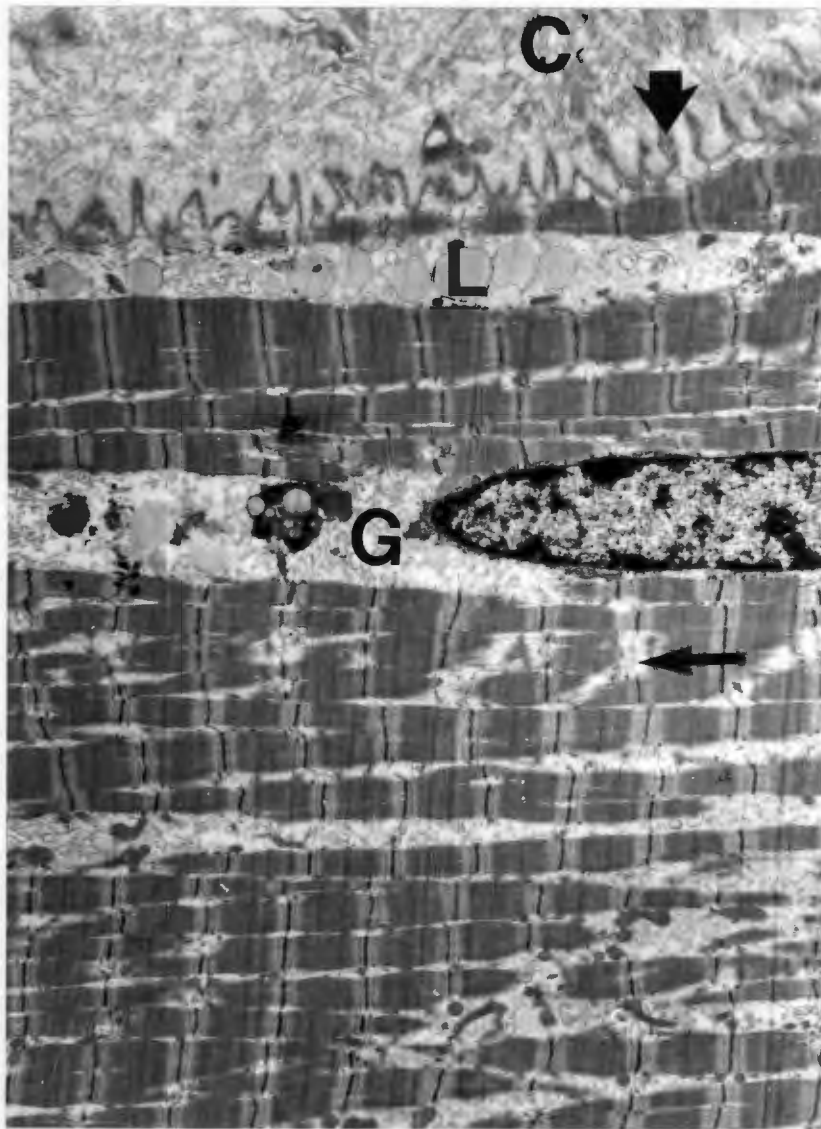


Fig 4.7. Electron micrograph showing a loss of myofilaments and z-bands (arrows), glycogen aggregates (G), subsarcolemmal lipid droplets (L), sarcolemmal folding (arrow head) and increased interstitial collagen (C). (x 10500)

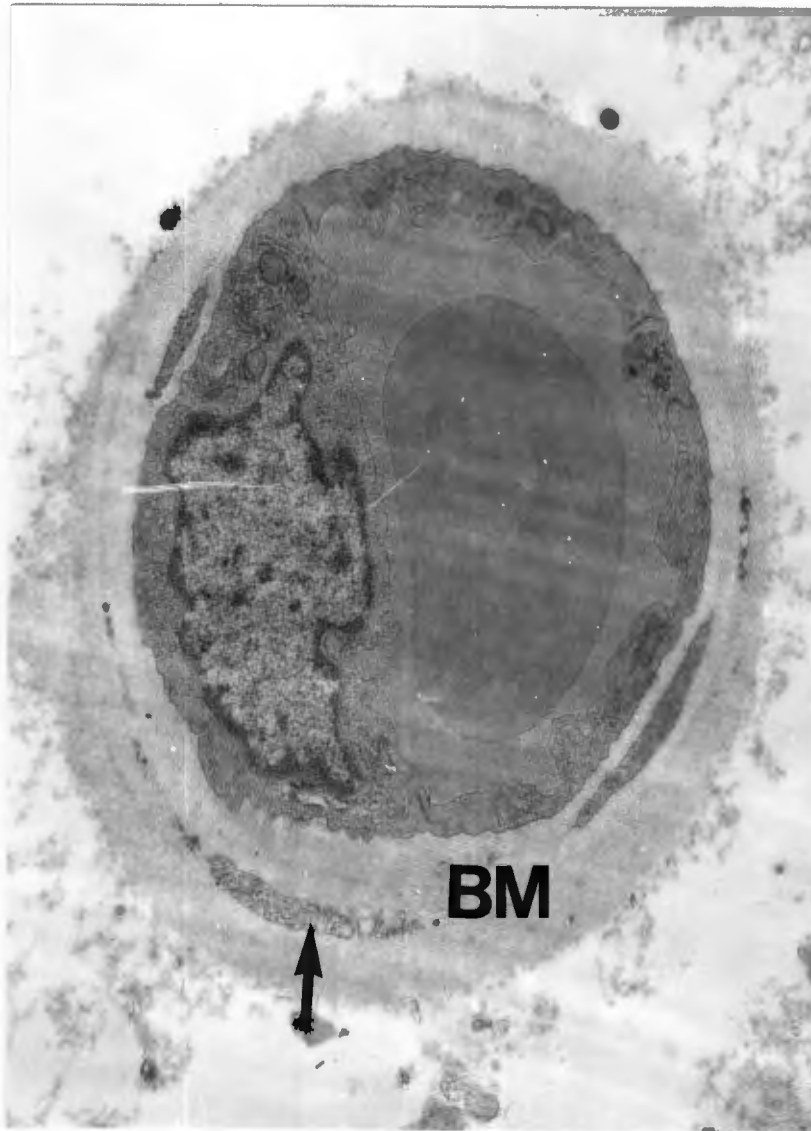


Fig 4.8. Electron micrograph of a capillary with a thickened, multilayered basement membrane (BM) containing debrinous material (arrow).

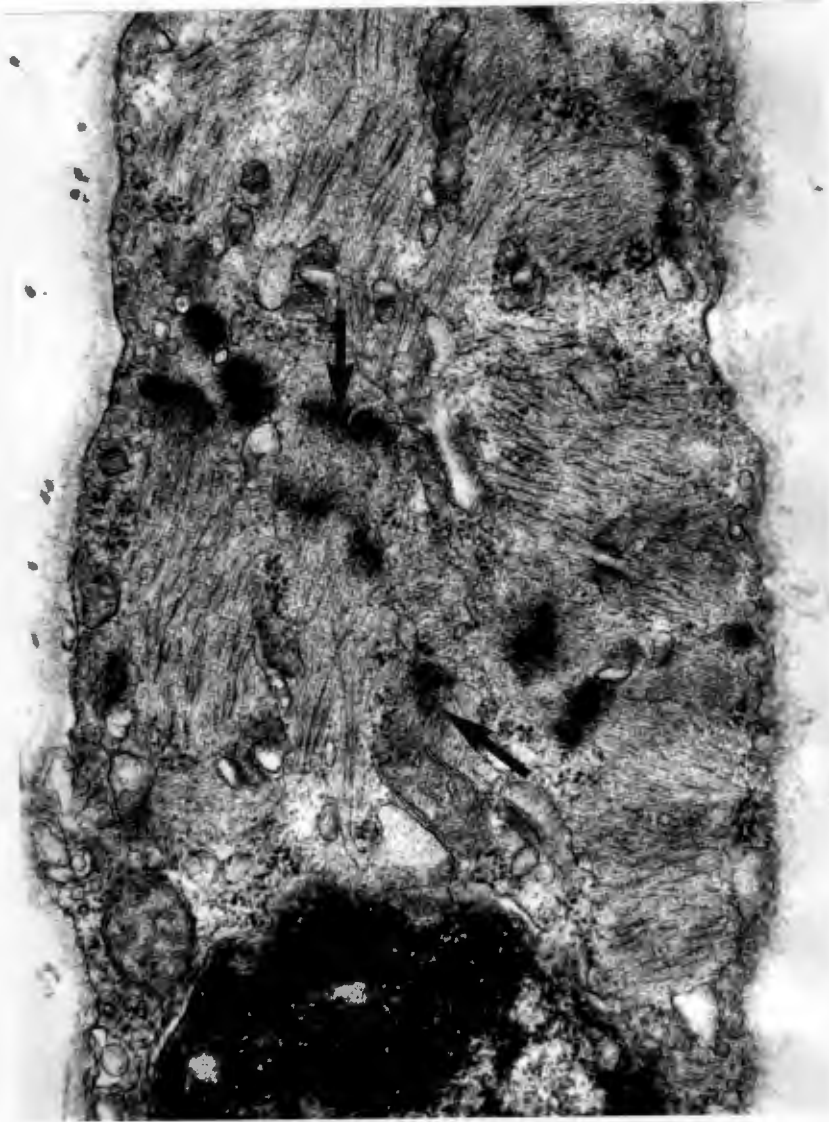


Fig 4.9. Electron micrograph of a myoblastic cell containing actin and myosin filaments, rudimentary z-bands (arrow) enveloped within a continuous basal layer. (x 44000)

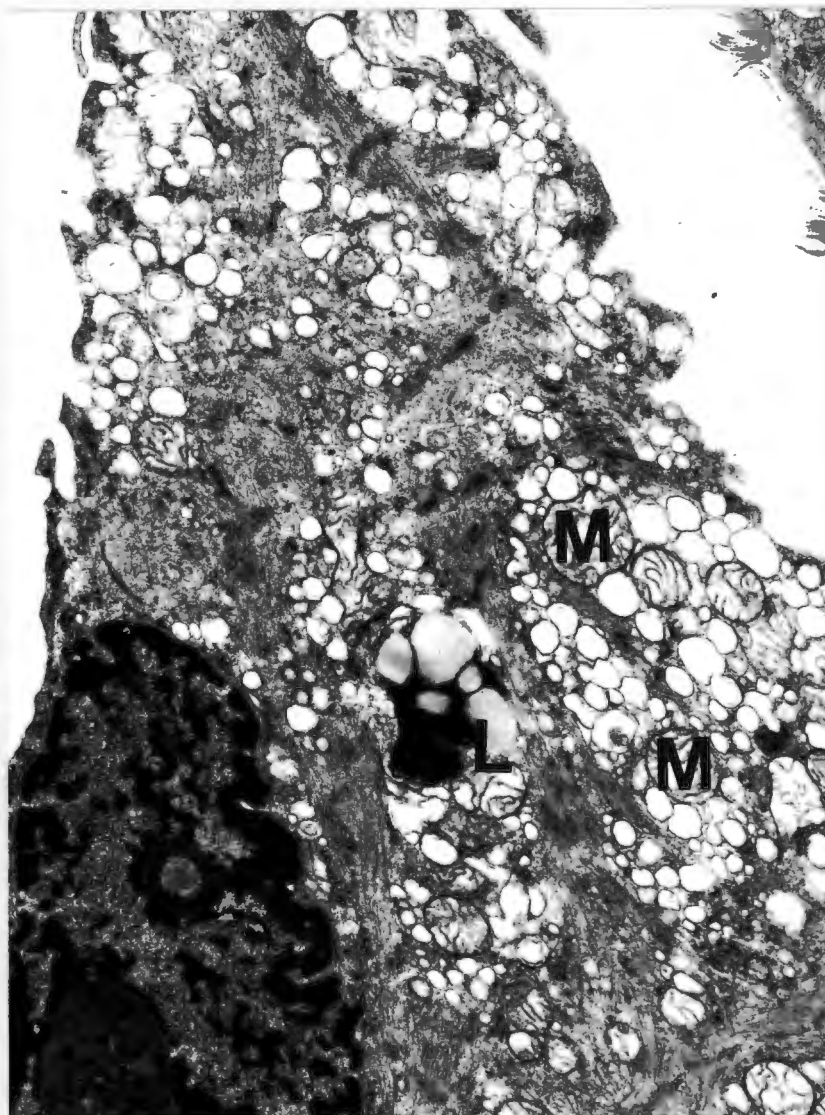


Fig 4.10. Electron micrograph showing a degenerate muscle cell with clumped chromatin, prominent nucleolus, disrupted cytoplasmic myofibrils, vacuolation, distended mitochondria (M) and lipofuscin (L). (x 22000)

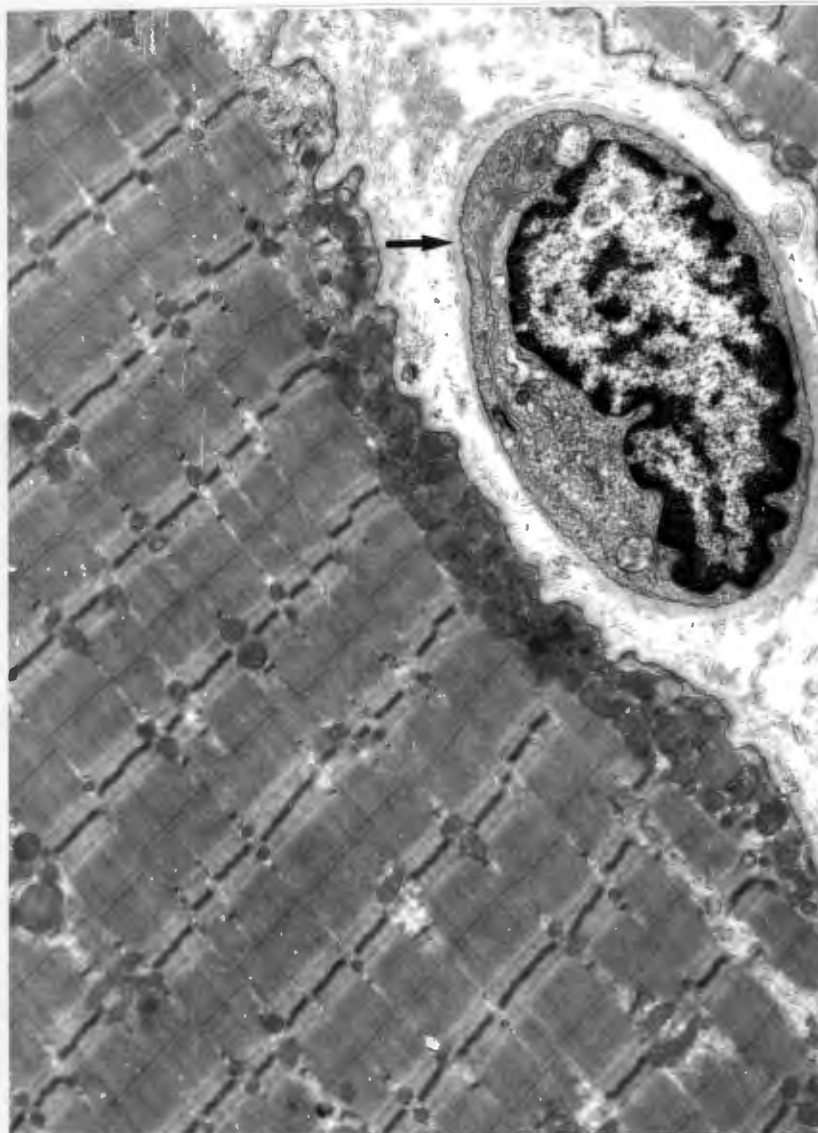


Fig 4.11 Electron micrograph of muscle from one of the controls showing normal sarcomeric units, mitochondria, and capillary basement membrane (arrow). (x 14000)

Electron microscopic analysis showed that of the samples from the eight control subjects, five had normal ultrastructural features (Figure 4.10); the myocytes contained smoothly outlined nuclei with evenly dispersed chromatin and nucleoli of normal size. The mitochondria were regular in shape and size, and small subsarcolemmal aggregates were occasionally seen. The myofibrils were aligned and the sarcomeric units showed regular A, Z, H, M and I banding. Glycogen granules were evenly dispersed throughout the cells and no large accumulations were present. The lipid droplets were of normal size and distribution, and were usually situated near the mitochondria. The sarcoplasmic membranes and overlying basement membranes were continuous and evenly contoured (Figure 4.11).

The muscle sample from one control subject revealed foci of myofibrillar loss and an occasional swollen mitochondrion, another had numerous lipid droplets, subsarcolemmal mitochondrial aggregates, and increased glycogen content while a third showed swollen mitochondria.

Ultrastructural morphometry of the controls revealed capillary basement membrane widths varying from 99 to 157 nm with a mean (\pm SEM) of 121 ± 3 nm ($p < 0.01$ vs. CHF).

Relationship of exercise performance to skeletal muscle pathology scores

The grading of the skeletal muscle biopsy specimens showed that patients with CHF had significantly higher scores for myopathic changes compared to the controls (12.2 ± 5.1 vs. 1.6 ± 2.0 arbitrary units; $p < 0.03$; *Table 4.5*).

Correlation analyses were performed between the skeletal muscle pathology score, ejection fraction and the other variables of peak exercise performance (described in Chapter 3) in the patients with CHF. *Table 4.5* shows that there was no significant correlation between the skeletal muscle pathology score and the resting ejection fraction, peak torque generated by the quadriceps muscles, MVC or time to fatigue during repetitive maximal isometric contraction/relaxation cycles. There was also no correlation between the ejection fraction and either WLpeak or VO₂ peak or any other tests of skeletal muscle function ($r = 0.12-0.32$; NS).

Table 4.4. Correlation coefficients for skeletal muscle pathology score, ejection fraction and peak exercise variables in patients with heart failure.

| Variable | r Value | P Value |
|---|---------|---------|
| Ejection fraction (%) | -0.30 | NS |
| VO ₂ peak (mlO ₂ /kg/min) | -0.64 | <0.05 |
| WLpeak (W) | -0.72 | <0.05 |
| PKTQ (Nm) | -0.5 | NS |
| PKTQ/LTV (Nm/cc) | -0.05 | NS |
| PKTH (Nm) | -0.59 | NS |
| PKTH/LTV (Nm/cc) | 0.53 | NS |
| TWQ (J) | -0.62 | <0.05 |
| TWQ/LTV (J/cc) | -0.58 | NS |
| TWH (J) | -0.49 | NS |
| TWH/LTV (J/cc) | -0.56 | NS |
| MVC (Nm) | -0.52 | NS |
| MVC/LTV (Nm/cc) | -0.60 | NS |
| TTF | -0.50 | NS |

Abbreviations: VO₂ peak, peak oxygen consumption; WLpeak, peak work load; PKTQ, peak torque of the quadriceps muscles; PKTH, peak torque hamstring muscles; W, watts; LTV, lean thigh volume, Nm, Newton metre; cc, cubic centilitre; TWQ, total work produced by the quadriceps muscles and (TWH) total work produced by the hamstring muscles during 25 maximal isokinetic contractions; J, joules; MVC, maximum voluntary contraction; TTF, time to fatigue.

However, a significant correlation was found between the pathology score and both the VO₂ peak ($r = -0.64$; $p < 0.05$), WLpeak ($r = -0.72$; $p < 0.05$) and total work performed by the quadriceps muscles during 25 maximal isokinetic contractions.

Discussion

The first important contribution of this study is to describe the nature of the changes which were prominent on light and electron microscopy in skeletal muscle samples in patients with severe CHF. Light microscopic changes included fibre splitting, fibre degeneration and regeneration, nuclear knots and chains, increased content of lipid droplets, and interstitial oedema. Fibre splitting, which was a consistent finding, has been identified in hypertrophied fibres and also in normal subjects following training, and is a sign of regeneration following muscle cell degeneration (Edgerton 1970). It has been hypothesized that the greater workload per remaining fiber and the greater associated metabolic stress may cause fibre splitting (Mancini et al. 1992; Francis et al. 1985). Since these patients are largely sedentary, these phenomena may be a consequence of prolonged inactivity of the skeletal muscles.

The presence of moth-eaten Type I fibres may be a reflection of a reduced oxidative capacity. In NADH preparations, moth-eaten fibres are seen with disruption and distortion of the intermyofibrillar network especially in zones of poor enzyme activity. Electron microscopy revealed mitochondrial and myofilamentous changes in these patients. Drexler et al. (1988) also reported a reduced number and size of mitochondria in patients with CHF, as well as a diminished number of mitochondrial cristae, a finding consistent with diminished oxidative enzyme capacity. These, as well as excessive intra-cellular lipid deposition and variation in muscle fibre areas found in our analysis are not specific to any single muscle disorder. Similar findings have been reported in physically-active renal patients studied in this laboratory (Diesel et al. 1993).

Filamentous bodies were found in two of the three patients with mitochondrial inclusions.

Teravanen and Makite (1977) found the proportion of mitochondrial myelin inclusions increased in proportion to the severity of clinical symptoms in patients with intermittent claudication. A similar relationship was found for decreased glycogen content, increased lipofuscin content and greater myofibrillar degeneration.

Severe capillary basement membrane thickening was also observed in these patients. Thickening of the basement membrane in capillaries in the pronator teres muscle (Longhurst et al. 1975) and in skin (Wroblewski et al. 1992) in patients with CHF shows that this disorder is widespread. Thickening of basement membranes has been described in patients with diabetes mellitus (Vracko 1970; Zacks et al. 1962), but the thickness of each membrane measured only between 80-200 nm

(Zacks et al. 1962), whereas in the present study, basement membrane thicknesses were in the range of 194 to 840 nm.

Atrophy of both Type I fibres (Lipkin et al. 1988; Sullivan 1990a; Poole-Wilson et al. 1988) and Type II fibres (Lipkin et al. 1988; Dunnigan et al. 1987; Poole-Wilson et al. 1988) have previously been reported in patients with CHF. We found that hypertrophy of both fibre types was also present and affected over 60% of all fibres in two patients.

Previous studies have reported an increased proportion of Type II fibres in patients with CHF (Sullivan 1990a; Lipkin et al. 1988; Mancini et al. 1989; Drexler et al. 1992). However, only two patients in this study showed Type II muscle fibre predominance and the difference in % Type II fibres between the groups was only 9%.

Whilst the above findings pertain to biopsy samples from the vastus lateralis muscle, similar histological abnormalities have been described in the pronator teres (Longhurst et al. 1975), deltoid and paraspinal (Smith et al. 1976), and diaphragmatic (Arora and Rochester 1982; Thurlbeck 1978) muscles in these patients. These findings suggest that this myopathic disorder is indeed widespread and involves both the large and small muscle groups. The mechanism underlying these structural abnormalities is unknown. Changes in skeletal muscle histology including Type I atrophy, fibre splitting, irregular shaped fibres, disintegrated fibrils, streaming z-lines, vacuolar degeneration and increased lipid deposits in the interfibrillar space have been described in patients after prolonged periods of immobilization (Appell 1990). Although it may be argued that the changes seen in the skeletal muscle of the patients in this study might be due to relative inactivity and deconditioning, it is important to consider that the patients in this study were only included if they were not bed-ridden and could participate in exercise testing. In addition, hypertrophy was a significant associated feature in many samples. Thus, the effects of prolonged bedrest were not assessed. Rather, our findings suggest that inactivity-induced atrophy cannot be the sole cause of skeletal muscle abnormalities in these patients. Poole-Wilson et al. (1988) reported that many histological abnormalities seen in patients with CHF are not typical of the changes seen as a result of either prolonged bed rest or of reversible ischaemia caused by peripheral vascular disease. Similar histological abnormalities have been described in asymptomatic physically active patients with hypertrophic cardiomyopathy (Smith et al. 1976) and with arrhythmias (Dunnigan et al. 1987). It is therefore probable that deconditioning alone is not responsible for the changes described in these patients.

It could be argued that the numerous medications ingested by the patients with CHF could also add to the abnormalities of skeletal muscle histology. However, similar structural changes in skeletal muscle have been described in asymptomatic young patients with cardiomyopathy who were not on any medications (Smith et al. 1976). It is therefore unlikely that our findings can be explained on the basis of a drug effect.

The second important finding was to show that whereas the ejection fraction, an index of central cardiovascular performance, could not predict the exercise capacity of these patients, the severity of skeletal muscle pathology significantly predicted exercise tolerance (*Table 4.4*). Others (Port et al. 1981, Baker et al. 1984) also found no significant relationship between the ejection fraction and exercise tolerance in patients with CHF.

The severity of the skeletal muscle pathology correlated best with exercise performance measured as WLpeak rather than VO₂ peak. This is also found in the healthy athletic population (Hawley and Noakes 1992). Drexler et al. (1992) have previously reported a significant relationship between peak exercise performance and decreased mitochondrial density and reduced surface area of mitochondrial cristae. These authors also reported that this relationship remained even when exercise performance improved with medication.

There was also no relationship between the ejection fraction and the severity of skeletal muscle pathology, highlighting the possibility that factors other than impaired central haemodynamic function may contribute to the myopathy.

In summary, this study provides evidence that severe skeletal muscle structural alterations exist and could perhaps contribute to the exercise intolerance in patients with CHF.

Conclusions

Future research should aim to determine the mechanism(s) and time course of these changes, so that effective management policies can be introduced to prevent the development and progression of the myopathy.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Summary

The first aim of this thesis was to evaluate the exercise capacity and skeletal muscle function in patients with CHF awaiting heart transplantation, and secondly to describe the histological and ultrastructural findings in skeletal muscle biopsy samples from these patients. The third aim was to develop a simple histological grading system to quantify the magnitude of changes in skeletal muscle and determine if a relationship exists between these changes and the exercise capacity of these patients.

The first study of this dissertation, reported in Chapter 3, established that the VO_2 peak, WL peak, and total work performed by the quadriceps and hamstring muscles in a 30 second isokinetic test, maximum voluntary contraction and time to fatigue during a test of isometric endurance were lower in patients with CHF compared with controls. Furthermore, patients with CHF stop exercising at lower heart rates, rates of O_2 consumption and ventilation, and at low blood lactate concentrations compared with control subjects. These findings are compatible with the interpretation that skeletal muscle limits the exercise tolerance of these patients but that this is not due to a more rapid increase in blood lactate concentrations in patients than in controls during exercise.

The study reported in Chapter 4 showed that significant histological and ultrastructural changes were found in all skeletal muscle biopsies from patients with CHF. These changes included atrophy and hypertrophy of fibres, fibre splitting, internalized nuclei, nuclear knots, moth-eaten fibres, increased lipid droplets. Electron microscopy showed a large variety of nonspecific abnormalities, including mitochondrial changes, Z-band degeneration and accumulation of intracellular glycogen. Ultrastructural morphometry revealed capillary basement membrane width significantly increased in the skeletal muscle of patients with CHF. A novel, blinded, impartially scored method for grading skeletal muscle pathology showed that skeletal muscle biopsies of patients with CHF had higher scores for myopathic changes compared to controls. Skeletal muscle pathology score correlated significantly with VO_2 peak, WLpeak, and TWQ, but not with EF%. EF% however did not correlate with either VO_2 peak, WLpeak or TWQ.

Conclusions

This thesis confirms that severe structural abnormalities exist in patients with CHF and may limit exercise capacity in these patients. In addition, patients with CHF have altered skeletal muscle function, including a decreased resistance to fatigue.

During graded exercise to exhaustion, patients with CHF seem to be limited by peripheral rather than by central, cardiovascular factors. This was suggested by the finding that graded exercise to exhaustion is terminated at lower heart rates, rates of O₂ consumption, ventilation and blood lactate concentrations in patients compared with controls.

All skeletal muscle biopsies from patients with CHF showed both histological and ultrastructural abnormalities.

Furthermore, the severity of the skeletal muscle pathology but not the resting systolic cardiac function, predicts exercise performance in patients with CHF. This finding highlights the possibility that factors other than impaired central haemodynamic function may contribute to the skeletal myopathy.

Future research should aim to determine the mechanism(s) and time course of these changes, so that effective management policies can be introduced to prevent the development and progression of the skeletal myopathy of patients with CHF.

CHAPTER 6

REFERENCES

- Adamopoulos S, Coats AJS. Peripheral abnormalities in chronic heart failure. *Postgrad Med J* 1991;67:S74-S80
- Alpert JS, Coffman JD, Baldimos MC, et al. Capillary permeability and blood flow in skeletal muscle of patients with diabetes mellitus and genetic prediabetes. *N Engl J Med* 1972;286:454-469
- Anderson EA, Mark AL. Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation* 1989;79:93-100
- Appell H-J. Muscular atrophy following immobilisation. *Sports Medicine* 1990;10:42-58
- Arnolda L, Conway M, Dolecki M, Sharif H, Rajagopalan B, Ledingham JGG et al. Skeletal muscle metabolism in heart failure: a ^{31}P nuclear magnetic resonance spectroscopy study of leg muscle. *Clin Sci* 1990;79:583-589
- Arora N, Rochester D. Effect of body weight and muscularity on human diaphragm muscle mass, thickness, and area. *J Appl Physiol* 1982;52:64-70
- Bain R, Bun Tan L, Murray RG. The correlation of cardiac power output to exercise capacity in chronic heart failure. *Eur J Appl Physiol* 1990;61:112-118
- Baker JB, Wilen MM, Body CM, Dinh H, Franciosa JA. Relation of right ventricular ejection fraction to exercise capacity in chronic left ventricular failure. *Am J Cardiol* 1984;54:596-599
- Ben Dov I, Sietsema KE, Casaburi R, Wasserman K. Evidence that circulatory oscillations accompany ventilatory oscillations during exercise in patients with heart failure. *Am Rev Respir Dis* 1992;145:776-781
- Benje W, Litchfield RL, Marcus ML. Exercise capacity in patients with severe left ventricular dysfunction. *Circulation* 1980;61:955-959
- Bergstrom J. Muscle electrolytes in man. *Scand J Clin Lab Invest* 1962;14(S68):1-47
- Bigland-Ritchie B, Furbish F, Woods JJ. Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors. *J Appl Physiol* 1986;61:421-429

Borg G, Holmgren A, Lindblad I. Perception of chest pain during physical work in a group of patients with angina pectoris. Stockholm, Sweden: Reports from the Institute of Applied Psychology 1980;81:1-8

Borg GAV. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;14:377-381

Braunwald E and Grossman W. Heart Disease. A textbook of cardiovascular medicine. Fourth Ed. WB Saunders Company. Harcourt Brace Jovanovich, Inc. 1992; p444.

Brussieres LM, Pflugfelder PW, Guiraudon C, Brown WF, Munoz DG, Taylor AW, Kostuk WJ. Exercise responses after cardiac transplantation in mitochondrial myopathy. *Am J Cardiol* 1993;71:1003-1006

Bruce RA. Exercise testing for evaluation of ventricular function. *N Engl J Med* 1977;296:671-675

Buller NP, Jones D, Poole-Wilson PA. Evidence for an intrinsic defect of skeletal muscle metabolism in patients with chronic heart failure. *Clin Sci* 1987;72:60P

Buller NP, Jones D, Poole-Wilson PA. Direct measurement of skeletal muscle fatigue in patients with chronic heart failure. *Br Heart J* 1991;65:20-24

Carr J, Stevenson L, Walden J, Heber D. Prevalence and hemodynamic correlates of malnutrition in severe congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 1989;63:709-713

Chapman SJ, Grindrod SR, Jones DA. Cross-sectional area and force production of the quadriceps muscle. *J Physiol (Lond)* 1984;353:53P

Cherniack NS, Longobardo GS. Cheyne-Stokes breathing, an instability in physiological control. *N Engl J Med* 1973;288:952-7

Clausen JP. Circulatory adjustments to dynamic exercise and effect of physical training in normal subjects and in patients with coronary artery disease. *Prog Cardiovasc Dis* 1976;28:459-495

Coats AJS, Adamopoulos S, Meyer T, Conway J, Sleight P. Physical training in chronic heart failure. *Lancet* 1990;335:63-6

Cody RJ, Kubo SH, Laragh JH, Atlas SA. Cardiac secretion of atrial natriuretic factor with exercise in chronic congestive heart failure patients. *J Appl Physiol* 1992;73:1637-1643

Coetzer P, Noakes TD, Sanders B, Lambert M, Bosch AN, Wiggins T, Dennis SC. Superior fatigue resistance of elite black South African distance runners. *J Appl Physiol* 1993;75:1822-1827

Cohn JN. Role of neurohumoral control of the circulation in determining exercise capacity in patients with heart failure. *Chest* 1992;101:347S-349S

Cohn JN, Archibald DG, Francis GS, Ziesche S, Franciosa JA, Harston WE, et al. Veterans Administration Cooperative Study on vasodilator therapy of heart failure: influence of prerandomization variables on the reduction of mortality by treatment with hydralazine and isosorbide dinitrate. *Circulation* 1987;75:IV49-IV54

Colucci WS, Ribiero JP, Rocco MB, Quigg R, Creager MA, Marsh JD, Gauthier DF, Hartley H. Impaired chronotropic response to exercise in patients with congestive heart failure. *Circulation* 1989;80:314-323

Conn EH, Whilom ORS, Wails GA. Exercise responses before and after physical conditioning in patients with severely depressed left ventricular function. *Am J Cardiol* 1982;49:296-300

Cowley AJ, Fullwood LJ, Muller AF, Stainer K, Skene AM, Hampton JR. Exercise capability in heart failure: is cardiac output important after all? *Lancet* 1991;337:771-773

Coyle EF, Martin WH, Sinacore DR, Joyner MJ, Hagberg JM, Holloszy JO. Time course of loss of adaptations after stopping prolonged intense endurance training. *J Appl Physiol* 1984;57:1857-1864

Criteria Committee, New York Heart Association. Diseases of the heart and blood vessels. Nomenclature and criteria for diagnosis 1964;6th Ed

Davies SW, Greig CA, Jordan SL, Lipkin DP. Short-stepping gait in severe heart failure. *Br Heart J* 1992;55:469-472

Dawson MJ, Gadian DG, Wilkie DR. Muscular fatigue investigated by phosphorus nuclear magnetic resonance. *Nature* 1978;274:861

Derman EW, Sims R, Noakes TD. The effects of antihypertensive medications on the physiological response to maximal exercise testing. *J Cardiovasc Pharmacol* 1991;18:S122-S127

Derman EW, Clark DR, Noakes TD. β 1-Selective blockade impairs training adaptations & the expected reduction of perceived exertion during exercise in hypertensive adults. *Europ Heart J* 1993;14:1328

Diesel W, Emms M, Knight B, Noakes TD, Swanepoel C, Van Zyl Smit R, Kaschula R, Sinclair-Smith C. Morphological features of the myopathy associated with chronic renal failure. *Am J Kid Dis* 1993;22:677-689

Diesel W, Noakes TD, Swanepoel C, Lambert M. Isokinetic muscle strength predicts maximal exercise tolerance in renal patients on chronic hemodialysis. *Am J Kidney Dis* 1990;XVI:109-114

Donald KW, Gloster J, Harris EA, Reeves J, Morris P. The production of lactic acid during exercise in normal subjects and patients with rheumatic heart disease. *Am Heart J* 1961;72:494-510

Drexler H, Munzel T, Riede U, Just H. Adaptive changes in the periphery and their therapeutic consequences. *Am J Cardiol* 1991b;67:29C-35C

Drexler H, Faude F, Hoing S, et al. Blood flow distribution within skeletal muscle during exercise in the presence of chronic heart failure: effect of milrinone. *Circulation* 1987a;76:1344-1352

Drexler H, Riede U, Hiroi M, Munzel T, Holubarsch C, Meinertz T. Ultrastructural analysis of skeletal muscle in chronic heart failure: relation to exercise capacity and indices of LV-dysfunction. *Circulation* 1988;78:107

Drexler H, Riede U, Munzel T, Konig H, Funke E, Just H. Alterations of skeletal muscle in chronic heart failure. *Circulation* 1992;85:1751-1759

Drexler H, Riede U, Schafer H-E. Reduced oxidative capacity of skeletal muscles in patients with severe heart failure. *Circulation* 1987b;76:178

Drexler H, Zeiher AM, Wollschlager H, Meinertz T, Just H, Bonzel T. Flow-dependent coronary artery dilatation in humans. *Circulation* 1989;80: in press.

Drexler H. Reduced exercise tolerance in chronic heart failure and its relationship to neurohumoral factors. *Eur Heart J* 1991a;12:21-28

Drexler H. Skeletal muscle failure in heart failure. *Circulation* 1992;85:1621-1623

Drexler H and Lu W. Endothelial dysfunction of hindquarter resistance vessels in experimental heart failure. *Am J Physiol* 1992;262:H1640-1645

Dubowitz V. *Muscle Biopsy- a practical approach*. 2nd edition:3-11. Bailliere Tindall, London 1985.

Dunnigan A, Staley NA, Smith SA, Pierpont ME, Judd D, Benditt DG, Benson DW. Cardiac and skeletal muscle abnormalities in cardiomyopathy: comparison of patients with ventricular tachycardia or congestive heart failure. *J Am Coll Cardiol* 1987;10:608-618

Durnin JVGA, Womersley J. Body fat assessed from the total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutrition* 1974;32:77-97

Edgerton VR. Morphology and histochemistry of the soleus muscle from normal and exercised rats. *Am J Anat* 1970;127:81-87

Edwards RHT, Toung A, Hosking GP, Jones DA. Human skeletal muscle function: Description of tests and normal values. *Clin Sci Mol Med* 1977;52:283-290

Eichna LW. Circulatory congestion and heart failure. *Circulation* 1960;22:864-886

Evans WJ, Phinney SD, Young VR. Suction applied to muscle biopsy maximizes sample size. *Med Sci Sports Exercise* 1982;14:101-102

Fink LI, Wilson JR, Ferraro N. Exercise ventilation and pulmonary artery wedge pressure in chronic stable congestive heart failure. *Am J Cardiol* 1986;57:249-53

Franciosa JA, Leddy CL, Wilen M, Schwartz D. Relation between haemodynamic and ventilatory response in determining exercise capacity in severe CHF. *Am J Cardiol* 1984;53:127-34

Franciosa JA, Park M, Levine B. Lack of correlation between exercise capacity and indexes of resting left ventricular performance in heart failure. *Am J Cardiol* 1981;47:33-39

Franciosa JA, Ziesche S, Wilen M. Functional capacity of patients with chronic left ventricular failure. Relationship of bicycle exercise performance to clinical and haemodynamic characterization. *Am J Med* 1979;67:460-466

Francis GS, Goldsmith SR, Ziesche S, Cohn JN. Response of plasma norepinephrine and epinephrine to dynamic exercise in patients with congestive heart failure. *Am J Cardiol* 1982;49:1152-6

Francis GS, Goldsmith SR, Ziesche S, Nakajima H, Cohn JN. Relative attenuation of sympathetic drive during exercise in patients with congestive heart failure. *J Am Coll Cardiol* 1985;5:832-839

Francis GS, Benedict C, Johnstone D, Kirlin P, Nicklas J, Liang C, Kubs S, Rudin-Toretsky E, Yusuf Y. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation* 1990;82:1724-1729

Garner SH, Sutton JR, Burse RL, McComas AJ, Cymerman A, Houston CS. Operation Everest II: Neuromuscular performance under conditions of extreme simulated altitude. *J Appl Physiol* 1990;1167-1172

Gibson JNA, Morrison WL, Halliday D, et al. Decrease in human quadriceps muscle protein turnover consequent upon leg mobilisation. *Clin Sci* 1987;72:503-509

Glanz SA, 1980. Biostatistics: how to detect, correct and prevent errors in the medical literature. *Circulation* 61:1-7

Green HJ. Manifestations and sites of neuromuscular fatigue. *Biochem Exerc* 1990;21:13-35

Gutman I, Wahlfeld AW. Lactate determination with lactated dehydrogenase and NAD. In: *Methods of enzymatic analysis*, edited by HV Bergmeyer. New York: Academic Press 1974:1464-1486.

Hanson P. Exercise testing and training in patients with chronic heart failure. *Med Sci Sports Exerc* 1994;26:527-537

Hawley JA, Noakes TD. Peak power output predicts maximal oxygen uptake and performance time in trained cyclists *Europ J Appl Physiol* 1992;65:79-83

Heath BH, Carter JEL. A modified somatotype method. *Am J Phys Anthropol* 1967;27:57-74

Higginbotham MB, Morris KG, Conn EH, Coleman RE, Cobb FR. Determinants of variable exercise performance among patients with severe left ventricular dysfunction. *Am J Cardiol* 1983;51:52-60

Hochachka PW, Dunn JF. Metabolic arrest: The most effective means of protecting tissues against hypoxia, in Sutton JR, Houston CS, Jones NL (eds): *Hypoxia, Exercise, and Altitude*. New York, Alan R Liss, 1983; 297-309

Hoffman A, Duba J, Lengyel M, Majer K. The effect of training on physical working capacity of MI patients with left ventricular dysfunction. *Eur Heart J* 1987;88:43-49

Jondeau G, Katz SD, Zohman L, Goldberger M, McCarthy M, Boudarias JP, LeJemtel TH. Active skeletal muscle mass and cardiopulmonary reserve. Failure to attain peak aerobic capacity during maximal bicycle exercise in patients with severe congestive heart failure. *Circulation* 1992;86:1351-1356

Karlsson J and Saltin B. Lactate, ATP, and CP in working muscles during exhaustive exercise in man. *J Appl Physiol* 1970;29:598

Karpati G, Carpenter S, Labrisseau A, Lafontaine R. The Kearns-Shy syndrome. A multidisease with mitochondrial abnormality demonstrated in skeletal muscle and skin. *J Neurosci* 1973;19:133-151

Kassis E. Baroreflex control of circulation in patients with congestive heart failure. Thesis. *Dan Med Bull* 1989;36:195-211

Katz S, Wahl J, Troha J. Specific phosphodiesterase inhibition and maximal and submaximal exercise performance in patients with congestive heart failure. *J Cardiovasc Pharm* 1989;14:S45-S48

Kavanagh T, Yacoub MH. Exercise after heart transplantation. *Ann Acad Med Singapore* 1992;21:372-378

Kellerman JJ, Ben-Ari E, Fisman E, Hayet M, Drory Y, Haimovitz D. Physical training in patients with ventricular impairment. *Adv Cardiol* 1986;34:131-147

Kellerman JJ, Shemesh J, Ben-Ari E. Contraindication to physical training in patients with impaired ventricular function. *Eur Heart J* 1988;9F:71-6

Kempeneers G, Myburgh KH, Wiggins T, Adams B, Van Zyl-Smit R, Noakes TD. Skeletal muscle factors limiting exercise tolerance of renal transplant recipients: Effect of a graded exercise training programme. *Am J Kidney Dis* 1990;14:57-65

Kempeneers G, Myburgh KH, Wiggins T, Adams B, Van Zyl-Smit R, Noakes TD. The effect of an exercise training program on renal transplant recipients. *Trans Proc* 1988;20:318-319

Kirlin PC, Grekin R, Das S, Ballor E, Johnson T, Pitt B. Neurohumoral activation during exercise in congestive heart failure. *Am J Med* 1986;81:623-629

Kitzman DW, Higginbotham MB, Cobb FR, Sheikh KH, Sullivan MJ. Exercise intolerance in patients with heart failure and preserved left ventricular systolic function: failure of the Frank-Starling mechanism. *J Am Coll Cardiol* 1991;17:1065-72

Kitzman DW, Sullivan MJ, Cobb FR, Higginbotham MB. Exercise cardiac output declines with advancing age in normal subjects. *J Am Coll Cardiol* 1989;13:241A

Koike A, Weiler-Ravell D, McKenzie DK, Zanconato S, Wasserman K. Evidence that the metabolic acidosis threshold is the anaerobic threshold. *J Appl Physiol* 1990;68:2521-2526

Kremser CB, O'Toole MF, Leff AR. Oscillatory hyperventilation in severe heart failure secondary to idiopathic dilated cardiomyopathy or ischemic cardiomyopathy. *Am J Cardiol* 1987;59:900-905

Kupper W. Interrupting the adaptive changes in congestive heart failure. *Am J Cardiol* 1991;67:20C-22C

Lange RL, Hecht HH. The mechanisms of Cheyne-Stokes respiration. *J Clin Invest* 1962;41:42-52

Leithe ME, Margorien RD, Hermiller JB, Unverferth DV, Leier CV. Relationship between central hemodynamics and regional blood flow in normal subjects and in patients with congestive heart failure. *Circulation* 1984;69:57

LeJemtel TH, Maskin CS, Lucido D, Chadwick CJ. Failure to augment maximal blood flow in response to one-leg versus two-leg exercise in patients with severe heart failure. *Circulation* 1986;74:245-251

Lerman A, Sandok EK, Hildebrand FL, Burnett JC Jr. Inhibition of endothelium-derived relaxing factor enhances endothelin-mediated vasoconstriction. *Circulation* 1992;85:1894-1898

Levine B, Kalman J, Mayer L, Fillit H, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med* 1990;323:236-241

Lewis T. Discussion of the heart. London: MacMillan 1933:1-2.

Lipkin DP, Jones DA, Round JM, Poole-Wilson P. Abnormalities of skeletal muscle in patients with chronic heart failure. *Int J Cardiol* 1988;18:187-195

Lipkin DP, Perrins J, Poole-Wilson PA. Respiratory gas exchange: the assessment of patients with impaired ventricular function. *Br Heart J* 1985;154:321-328

Longhurst J, Capone R, Zelis R. Evaluation of skeletal muscle capillary basement membrane thickness in congestive heart failure. *Chest* 1975;67:195-198

Longhurst J, Gifford W, Zelis R. Impaired forearm oxygen consumption during static exercise in patients with congestive heart failure. *Circulation* 1976;54:477-480

Mack G, Nose H, Nadel ER. Role of cardiopulmonary baroreflexes during dynamic exercise. *J Appl Physiol* 1988;65:1827-1832

Magnusson G, Isberg B, Karlberg K, Sylven C. Skeletal muscle strength and endurance in chronic congestive heart failure secondary to idiopathic dilated cardiomyopathy. *Am J Cardiol* 1994;73:307-309

Mancini D, Coyle E, Ferraro N. Skeletal muscle metabolic abnormalities in heart failure are in part due to intrinsic skeletal muscle changes. *J Am Coll Cardiol* 1989;13:39A

Mancini DM, Ferraro N, Tuchler M, Chance B, Wilson JR. Detection of abnormal calf muscle metabolism in patients with heart failure using phosphorus-31 nuclear magnetic resonance. *Am J Cardiol* 1988;62:1234-1240

Mancini DM, Wilson JR, Bolinger L, Li H, Kendrick K, Chance B, Leigh JS. In vivo magnetic resonance spectroscopy measurement of deoxymyoglobin during exercise in patients with heart failure - demonstration of abnormal muscle metabolism despite adequate oxygenation. *Circulation* 1994; 90:500-508

Mancini DM, Davis L, Wexler JP, Chadwick B, LeJemtel TH. Dependence of enhanced maximal exercise performance in increased peak skeletal muscle perfusion during long term captopril in heart failure. *J Am Coll Cardiol* 1987;10:845-850

Mancini DM, Eisen H, Kussmaul W, Mull R, Edmunds LH, Wilson JR. Value of peak oxygen consumption for optimal timing of cardiac transplantation in ambulatory patients with heart failure. *Circulation* 1991a;83:778-786

Mancini DM, Ferraro N, Nazzaro D, Chance B, Wilson JR. Respiratory muscle deoxygenation during exercise in patients with heart failure demonstrated with near-infrared spectroscopy. *J Am Coll Cardiol* 1991b;18:492-498

Mancini DM, Walter G, Reichel N, Lenkinski R, McCully KK, Mullen JL, Wilson JR. Contribution of skeletal muscle atrophy to exercise intolerance and altered muscle metabolism in heart failure. *Circulation* 1992;85:1364-1373

Margulies K, Perrella M, McKinley L, Burnett JC Jr. Angiotensin inhibition potentiates the renal responses to neutral endopeptidase inhibition in dogs with congestive heart failure. *J Clin Invest* 1991;88:1636-1648

Marie PY, Escanye JM, Brunotte F, Robin B, Walker P, Zannad F, Robert J, Gilgenkrantz JM. Skeletal muscle metabolism in the leg during exercise in patients with congestive heart failure. *Clin Sci* 1990;78:515-519

Martin AD, Spenst LF, Drinkwater DT, et al. Anthropometric estimation of muscle mass in men. *Med Sci Sports Exerc* 1990;22:729-733

Martin WH, Berman WI, Buckey JC, Snell PG, Blomqvist CG. Effects of active muscle mass size on cardiopulmonary responses to exercise in congestive heart failure. *J Am Coll Cardiol* 1989;14:683-694

Marzo KP, Herrmann HA, Rein A, Mancini D. Acute effect of balloon mitral valvuloplasty on exercise capacity, ventilation and skeletal muscle oxygenation. *Circulation* 1991;84:II-72

Marzo KP, Wilson JR, Mancini DM. Effects of cardiac transplantation on ventilatory response to exercise. *Am J Cardiol* 1992;69:547-53

Maskin CS, Forman R, Sonnenblick EH, Frishman WH, LeJemtel TH. Failure of dobutamine to increase exercise capacity despite haemodynamic improvement in severe chronic heart failure. *Am J Cardiol* 1983;51:177-182

Massie BM, Conway M, Yonge R, Frostick S, Ledingham J, Sleight P, Radda G, Rajagopalan B. Skeletal muscle metabolism during exercise under ischemic conditions in congestive heart failure. *Circulation* 1988;78:320-326

Massie BM, Conway M, Yonge R, Frostick S, Ledingham J, Sleight P, Radda G, Rajagopalan B. Skeletal muscle metabolism in patients with congestive heart failure: relation to clinical severity and blood flow. *Circulation* 1987a;76:1009-19

Massie BM, Conway M, Yonge R, Frostick S, Sleight P, Ledingham J, Radda G, Rajagopalan B. 31-P nuclear magnetic resonance evidence of abnormal skeletal muscle metabolism in patients with congestive heart failure. *Am J Cardiol* 1987b;60:309-315

Massie BM. Exercise tolerance in congestive heart failure: Role of cardiac function, peripheral blood flow, and muscle metabolism and effect of treatment. *Am J Med* 1988;84:75-82

Maughan RJ. Relationship between muscle strength and muscle cross-sectional area. *Sports Med* 1984;1:263-269

McGrath BP, Arnold L, Matthews PG, Jackson B, Jennings G, Kiat H, Johnston CI. Controlled trial of enalapril in congestive heart failure. *Br Heart J* 1985;54:405-414

McMurray J, Abdullah I, Dargie HJ, Shapiro D. Increased concentrations of tumour necrosis factor and "cachectic" patients with severe chronic heart failure. *Br Heart J* 1991;66:356-358

Meakins J, Long CNH. Oxygen consumption, oxygen debt and lactic acid in circulatory failure. *J Clin Invest* 1927;4:273-293

Meiler SE, Ashton JJ, Moeschberger ML, Unverferth DV, Leier CV. An analysis of the determinants of exercise performance in congestive heart failure. *Am Heart J* 1987;113:1207-17

Minotti JR, Pillay P, Oka R, Wells L, Christoph I, Massie BM. Skeletal muscle size: relationship to muscle function in heart failure. *J Appl Physiol* 1993;75:373-381

Minotti JR, Christoph I, Oka RK, Weiner MW, Wells L, Massie BM. Impaired skeletal muscle function in patients with congestive heart failure: relationship to systemic exercise performance. *J Clin Invest* 1991a;88:2077-2082

Minotti JR, Christoph I, Oka RK, Weiner MW, Wells L, Massie BM. Significance of muscle atrophy in heart failure. *Circulation* 1991b;84:597

Minotti JR, Johnson EC, Hudson TL, Zuroske G, Murata G, Fukushima E, Cagle TG, Chick TW, Massie BM, Icenogle MV. Skeletal muscle response to exercise training in congestive heart failure. *J Clin Invest* 1990;86:751-758

Minotti JR, Pillay P, Chang L, Wells L, Massie BM. Neurophysiological assessment of skeletal muscle fatigue in patients with congestive heart failure. *Circulation* 1992;86:903-908

Miyagi K, Asanoi H, Ishizaka S, Kameyama T, Sasayama S. Limited value of anaerobic threshold for assessing functional capacity in patients with heart failure. *Clin Cardiol* 1993;16:133-137

Moore DP, Weston AR, Hughes JMB, Oakley CM, Cleland JGF. Effects of increased inspired oxygen concentrations on exercise performance in chronic heart failure. *Lancet* 1992;339:850-953

- Morrison WL, Gibson JNA, Johnston RN, Yacoub M, Rennie MJ. Improved whole body protein turnover following heart and lung transplantation. *Lancet* 1988;ii:853-854
- Mudge GH, Goldstein S, Addonizio LJ, Caplan A, Mancini D, Levine TB, Ritsch ME, Stevenson LW. Task Force 3: Recipient Guidelines/ Prioritization. *J Am Coll Cardiol* 1993;22:21-64
- Muller AF, Batin P, Evans S, Hawkins M, Cowley AJ. Regional blood flow in chronic heart failure: the reason for the lack of correlation between patients' exercise tolerance and cardiac output? *Br Heart J* 1992;67:478-481
- Musch TI, Moore RL, Leathers DJ, Bruno A, Zelis R. Endurance training in rats with chronic heart failure induced by myocardial infarction. *Circulation* 1986;74:431-441
- Myers J, Froelicher VF. Hemodynamic determinants of exercise capacity in chronic heart failure. *Ann Internal Med* 1991;115:377-386
- Myers J, Salleh A, Buchanan N, Smith D, Neutel J, Bowes E, Froelicher VF. Ventilatory mechanisms of exercise intolerance in chronic heart failure. *Am Heart J* 1992;124:710
- Nishimura Y, Maeda H, Tanaka K, Nakamura H, Hashimoto Y, Yokoyama M. Respiratory muscle strength and haemodynamics in chronic heart failure. *Chest* 1994;105:355-359
- Noakes TD, Myburgh K, Schall R. Peak treadmill running velocity during VO_2 max test predicts running performance. *J Sports Sci* 1990;8:35-45
- Noakes TD. Implications of exercise testing for prediction of athletic performance: a contemporary perspective. *Med Sci Sports Exerc* 1988;20:319-330
- Noble BJ. Clinical applications of perceived exertion. *Med Sci Sports Exerc* 1982;14:406-411
- Olson W, Engel WK, Walsh GO, Einaugler R. Oculocraniosomatic neuromuscular disease with 'ragged red' fibres. *Arch Neurol Exp Neurol* 1972;26:193-211
- Packer M. Abnormalities of diastolic function as a potential cause of exercise intolerance in chronic heart failure. *Circulation* 1990;81:78-86

- Packer M. Neurohormonal interaction and adaptation in chronic heart failure. *Circulation* 1988;77:721-30
- Packer M. Pathophysiology of chronic heart failure. *Lancet* 1992a;340:88-92
- Packer M. Treatment of chronic heart failure. *Lancet* 1992b;340:91-95
- Patterson JA, Naughton J, Peitras RM. Treadmill exercise in assessment of the functional capacity of patients with cardiac disease. *Am J Cardiol* 1972;30:757-762
- Peterson H, Rothschild M, Weinberg C, Fell R, McLeisch K, Pfeifer M. Body fat and the activity of the autonomous nervous system. *N Engl J Med* 1988;318:1077-1083
- Pfeffer MA, Braunwald E, Moyle LA, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the Survival and Ventricular Enlargement Trial. *N Engl J Med* 1992;327:669-677
- Poole-Wilson PA, Nigel PB, Lipkin DP. Regional blood flow, muscle strength and skeletal muscle histology in severe congestive heart failure. *Am J Cardiol* 1988;62:49E-52E
- Port S, McEwan P, Cobb FR, Jones RH. Influence of right ventricular function on left ventricular response to exercise in patients with coronary artery disease. *Circulation* 1981; 63:856-863
- Rajagopalan B, Conway MA, Massie B, Radda GK. Alterations of skeletal muscle metabolism in humans studied by phosphorus 31 magnetic resonance spectroscopy in congestive heart failure. *Am J Cardiol* 1988;62:53E-57E
- Ralston MA, Merola AJ, Leier CV. Depressed aerobic enzyme activity of skeletal muscle in severe chronic heart failure. *J Lab Clin Med* 1991;117:370-2
- Reddy HK, Weber KT, Janicki JS, McElroy PA. Light isometric exercise in patients with chronic heart failure. *J Am Coll Cardiol* 1988;12:353-358
- Ribiero JP, Knutzen A, Rocco MB, Hartley LH, Colucci WS. Periodic breathing during exercise in severe heart failure. *Chest* 1987;92:555-6

Riley M, Elborn JS, Bell N. Substrate utilization during exercise in chronic cardiac failure. *Clin Sci* 1990;79:89-95

Rodeheffer R, Lerman A, Heublein DH, Burnett JC Jr. Plasma endothelin concentrations are increased in humans with congestive heart failure. *Mayo Clin Proc* 1992;67:719-724

Roubin GS, Anderson SD, Shen WF, Choong CY, Alwyn M, Hillery S, Harris P, Kelly DT. Haemodynamic and metabolic basis of impaired exercise tolerance in systemic LV dysfunction. *J Am Coll Cardiol* 1990;15:986-994

Rubin S, Swan HJC. Response to exercise of patients with severe chronic heart failure. *Bibliotheca Cardiol* 1986;40:52-60

Saltin B, Gollnick PD. Skeletal muscle adaptability: Significance for metabolism and performance, in *Handbook of Skeletal Muscle*. Bethesda, Md, American Physiological Society 1988;589-596

Sapega AA, Heppenstall RB, Chance B, Park YS, Sokolow D. Optimizing tourniquet application and release times in extremity surgery. *J Bone Joint Surg* 1985;67-A:303-314

Schantz P, Randall-Fox E, Hutchinson W, Tyden A, Astrand PO. Muscle fibre type distribution, muscle cross sectional area and maximal voluntary strength in humans. *Acta Physiol Scand* 1983;117:219-226

Simonton CA, Higginbotham MB, Cobb FR. The ventilatory threshold: Quantitative analysis of reproducibility and relation to arterial lactate concentration in normal subjects and in patients with chronic congestive heart failure. *Am J Cardiol* 1988;62:100-107

Sinoway LI, Minotti JR, Dwight D, Pencock JL, Burg JE, Musch TI, Zelis R. Delayed reversal of impaired vasodilation in congestive heart failure after heart transplantation. *Am J Cardiol* 1988;61:1076-1079

Smith ER, Heffernan LP, Sangalang VE, Vaughan LM, Flemington CS. Voluntary muscle involvement in hypertrophic cardiomyopathy. *Ann Int Med* 1976;85:556-572

SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med* 1992;327:685-691

Spierstein MD, Unger RH, Madison LL. Studies of muscle capillary basement membranes in normal subjects, diabetic, and prediabetic patients. *J Clin Invest* 1968;47:1973-1999

Stingo A, Clavell A, Aarhus L, Burnett JC Jr. Cardiovascular and renal actions of C-type natriuretic peptide. *Am J Physiol* 1992;262:H308-H312

Strandell T, Shepherd JT. The effect in humans of increased sympathetic activity on the blood flow to active muscles. *Acta Med Scand Suppl* 1967;472:146-167

Stratton JR, Dunn JF, Adamopoulos S, Kemp GJ, Coats AJS, Rajagopalan B. Training partially reverses skeletal muscle metabolic abnormalities during exercise in heart failure. *J Appl Physiol* 1994;76(4):1575-1582

Sullivan J, Saltin B, Klitgaard H, Cobb FR. Altered expression of myosin heavy chain isoforms in skeletal muscle in patients with chronic heart failure. *Circulation (abstr)* 1990b;82:4

Sullivan MJ, Green HJ, Cobb FR. Skeletal muscle biochemistry and histology in ambulatory patients with long-term heart failure. *Circulation* 1990a;81:518-27

Sullivan MJ, Higginbotham MB, Cobb FR. Increased exercise ventilation in patients with chronic heart failure: intact ventilatory control despite hemodynamic and pulmonary abnormalities. *Circulation* 1988b;77:552-559

Sullivan MJ, Higginbotham MB, Cobb FR. Exercise training in patients with severe left ventricular dysfunction: haemodynamic and metabolic effects. *Circulation* 1988a;78:506-515

Sullivan MJ, Higginbotham MB, Green HJ, Cobb FR. Exercise skeletal muscle energetics in heart failure: accelerated glycogenolysis and glycolysis and an altered maximal metabolic end-point. *Circulation* 1988c;78:107

Sullivan MJ, Green HJ, Cobb FR. Altered skeletal muscle metabolic response to exercise in chronic heart failure. *Circulation* 1991;84:1597-1607

Sullivan MJ, Knight JD, Higginbotham MB, Cobb FR. Relation between central and peripheral haemodynamics during exercise in patients with chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion pressure. *Circulation* 1989;80:769-81

Sullivan MJ, Merola AJ, Timmerman AP, Unverferth DV, Leier CV. Drug-induced aerobic enzyme activity of human skeletal muscle during bedrest deconditioning. *J Cardiopulm Rehab* 1986;6:232-237

Swan HJ. Significance and prognostic value of left ventricular function in ischaemic heart disease and in cardiomyopathy. *Adv Cardiol* 1986;34:45-57

Sylvan JM, Higginbotham BM, Cobb FAR. Exercise training in patients with severe left ventricular dysfunction: haemodynamic and metabolic effects. *Circulation* 1988;78:506-515

Szlachcic J, Massie BM, Kramer BL, Topic N, Tubau J. Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. *Am J Cardiol* 1985;55:1037-42

Teravanen H, Makitie J. Striated muscle ultrastructure in intermittent claudication. *Arc Pathol Lab Med* 1977;101:230-235

Thompson JM, Dempsey JA, Chosy LW, Shahidi NT, Reddan WG. Oxygen transport and oxyhaemoglobin dissociation during prolonged muscular work. *J Appl Physiol* 1974; 37:658-64

Thurlbeck W. Diaphragm and body weight in emphysema. *Thorax* 1978;33:483-48

Toussaint D, Dustin P. Electron microscopy of normal and diabetic retinal capillaries. *Arch Ophthalmol* 1963;70:96

Vracko R. Skeletal muscle capillaries in diabetics: A quantitative analysis. *Circulation* 1970;41:271

Wada O, Hidetsugu A, Miyagi K, Ishizaka S, Kameyama T, Seto H, Sasayama S. Importance of abnormal lung perfusion in excessive exercise ventilation in chronic heart failure. *Am Heart J* 1992;125:790-798

Wade OL, Bishop JM. Cardiac output and regional blood flow. Blackwell, Oxford. 51-72 1962.

Walker PM, Idstrom JP, Shiersten T, Bylund-Fellenius AC. Oxygen dependence of energy metabolism in contracting and recovery rat skeletal muscle. *Am J Physiol* 1985;248:H40

Weber KT, Janicki JS. Lactate production during maximal and submaximal exercise in patients with chronic heart failure. *J Am Coll Cardiol* 1985b;6:717-724

Weber KT, Janicki JS. Cardiopulmonary exercise testing for evaluation of chronic heart failure. *Am J Cardiol* 1985a;55:22A-31A

Weber KT, Kinasewitz GT, Janicki JS, Fishman AP. Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. *Circulation* 1982;65:1213-1223

Weiss S, Ellis LB. Oxygen utilization and lactic acid production in the extremities during rest and exercise. *Arch Intern Med* 1935;55:665-680

Wiener DH, Fink LI, Maris J, Jones RA, Chance B, Wilson JR. Abnormal skeletal muscle bioenergetics during exercise in patients with heart failure: Role of reduced muscle blood flow. *Circulation* 1986;73:1127-1136

Wilson JR, Ferraro N. Exercise intolerance in patients with chronic heart failure: Relation to oxygen transport and ventilatory abnormalities. *Am J Cardiol* 1983;51:1358-1363

Wilson JR, Fink L, Maris J, Ferraro N, Power-Vanwart J, Eleff S, Chance B. Evaluation of skeletal muscle energy metabolism in patients with heart failure with gated phosphorus-31 nuclear magnetic resonance. *Circulation* 1985;71:57-62

Wilson JR, Frey MJ, Mancini DM, et al. Sympathetic vasoconstriction during exercise in ambulatory patients with left ventricular failure. *Circulation* 1989;79:1021-1027

Wilson JR, Mancini DM, Simson M. Detection of skeletal muscle fatigue in patients with heart failure using electromyography. *Am J Cardiol* 1992;70:488-493

Wilson JR, Martin JL, Ferraro N, Weber KT. Effect of hydralazine on perfusion and metabolism in the leg during upright bicycle exercise in patients with heart failure. *Circulation* 1983;69:425-32

Wilson JR, Martin JL, Ferraro N. Impaired skeletal muscle nutritive flow during exercise in patients with congestive heart failure: role of cardiac pump dysfunction as determined by the effect of dobutamine. *Am J Cardiol* 1984a;53:1308-1315

Wilson JR, Martin JL, Schwartz MD, Ferraro N. Exercise intolerance in patients with chronic heart failure: role of impaired nutritive flow to skeletal muscle. *Circulation* 1984b;69:1079-87

Wroblewski H, Kastrup J, Norgaard T, Mortensen S, Haunso S. Evidence of increased microvascular resistance and arteriolar hyalinosis in skin in congestive heart failure secondary to idiopathic dilated cardiomyopathy. *Am J Cardiol* 1992;69:769-774

Yamabe H, Itoh K, Yasaka Y, Kakimoto T, Yokoyama M. The distribution of the blood flow during exercise in chronic heart failure - compensatory mechanism to the decreased cardiac output. *Japanese Circulation J - Eng Ed.* 1992;56:494-499

Yancy CW, Parsons D, Lane L, Carry M, Firth B, Blomquist G. Capillary density, fiber type and enzyme composition of skeletal muscle in congestive heart failure. *J Am Coll Cardiol* 1989;13:38A

Zacks SI, Pegues JJ, Elliot FA. Interstitial muscle capillaries in patients with diabetes mellitus: A light and electron microscope study. *Metab* 1962;11:381

Zelis R, Longhurst J, Capone RJ, Mason DT. A comparison of regional blood flow and oxygen utilization during dynamic forearm exercise in normal subjects and patients with congestive heart failure. *Circulation* 1974; 50:137-143

Zelis R, Flaim SF. Alterations in vasomotor tone and congestive heart failure. *Prog Cardiovasc Dis* 1982;24:437-459

Zelis R, Mason DT, Braunwald E. A comparison of the effects of vasodilator stimuli on peripheral resistance vessels in normal subjects and in patients with congestive heart failure. *J Clin Invest* 1968;47:960-970

Zelis R, Nellis SH, Longhurst J, Lee G, Mason DT. Abnormalities in the regional circulations accompanying congestive heart failure. *Prog Cardiovasc Dis* 1975;18:181-209

Zelis R, Sinoway L, Musch T. Why do patients with congestive heart failure stop exercising? Ed comment. *J Am Coll Cardiol* 1988;12:359-361