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Cardiorespiratory And Peripheral Adaptations After Cardiac Transplantation

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CARDIORESPIRATORY AND PERIPHERAL ADAPTATIONS AFTER CARDIAC TRANSPLANTATION

by

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ABSTRACT

The cardiovascular, pulmonary and musculoskeletal systems were investigated in seven studies of patients with end-stage heart failure before and/or after receiving a heart transplant. The purposes of the studies were to determine the time course and magnitude of the changes in peak oxygen uptake, cardiopulmonary response to exercise and peripheral skeletal muscle histology and biochemistry after cardiac transplantation. In addition, the physiological basis for the limited exercise capacity and the effect of exercise posture on cardiac performance and secretion of atrial natriuretic peptide was also evaluated in cardiac transplant patients.

In the first study, the peak oxygen uptake almost doubled from the pre-transplant values in the first three months following transplantation. The increased exercise capacity was associated with improved blood pressure, heart rate and ventilatory responses to exercise. In the second study, lung volumes, but not diffusing capacity, improved in the first year after cardiac transplantation. In the third study, increased skeletal muscle fibre cross sectional area and glycolytic and oxidative enzyme activities were observed in the first year post transplantation using skeletal muscle biopsy of the vastus lateralis.

In the fourth study, the low peak oxygen uptake, as a percent of predicted, in patients after cardiac transplantation appears to be due to some peripheral limitations.

In the fifth study, the marked increase in cardiac filling pressure during supine exercise was associated with a lower slope of the cardiac output-oxygen uptake relationship when compared to the upright position. In the sixth study, higher cardiac filling pressures at peak supine exercise were associated with

higher plasma ANP levels when compared to exercise in the upright posture. In the final study, two patients with mitochondrial myopathy who underwent cardiac transplantation demonstrated the typical hyperkinetic circulatory response to exercise despite cardiac denervation.

The present results show that exercise capacity improves markedly early after cardiac transplantation. The improved exercise capacity is associated with changes in lung function and peripheral skeletal muscle adaptations in the first year after transplantation. The exercise capacity however is still low at one or more years following transplantation (2/3 of predicted) possibly due to peripheral limitations.

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LIST OF ABBREVIATIONS

ANP ..... atrial natriuretic peptide
AT ..... ventilatory threshold
ATPase ..... adenosine triphosphatase
a-v O_2 diff ..... arterio-venous oxygen difference
BP sys ..... systolic blood pressure
BP dias ..... diastolic blood pressure
CI ..... cardiac index
CO ..... cardiac output
Co_2 ..... carbon dioxide
Cp ..... capillaries
CS ..... citrate synthase
CSA ..... cross sectional area
DLco ..... diffusing capacity
FAI ..... functional aerobic impairment
FEV_1 ..... forced expired volume at 1 second
FEV_1/FVC ..... ratio of forced expired volume at 1 second and forced vital capacity
FRC ..... functional residual capacity
FVC ..... forced vital capacity
HAD ..... ß-hydroxyacyl CoA dehydrogenase
IC ..... inspiratory capacity
irANP ..... immunoreactive atrial natriuretic peptide
Kco ..... diffusing capacity corrected for alveolar volume
NADH ..... nicotinamide adenine dinucleotide
NYHA ..... New York Heart Association
m ..... metre
mo ..... month
PAP mean ..... mean pulmonary artery pressure
PCWP ..... pulmonary capillary wedge pressure
PEFR ..... peak expiratory flow rate

PFK ..... phosphofructokinase
PVR ..... pulmonary vascular resistance
RAP ..... right atrial pressure
RRM ..... respiratory rate per minute
RV ..... residual volume
sec ..... second
SEM ..... standard error of the mean
SVR ..... systemic vascular resistance
TLC ..... total lung capacity
TV ..... tidal volume
VCO_2 ..... carbon dioxide output
V_E ..... minute ventilation
V_E/VCO_2 ..... relation between minute ventilation and carbon dioxide output
VO_2 ..... oxygen uptake
VO_2 max ..... peak oxygen uptake
ww ..... wet weight

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gases were sampled continuously (rate 4-6 L/min) and analyzed for O_2 and CO_2 content (Ametek). Data were processed on line by a microcomputer programmed to correct for delays between the signals for volume and gas concentration. Average values for each 15 seconds interval were presented for the following variables: respiratory rate, minute ventilation, tidal volume, oxygen uptake, carbon dioxide output, and heart rate. The anaerobic threshold was determined non-invasively using the V-slope method (Beaver et al., 1986) with the mathematical calculation performed by the computer. Individual slopes of the relationship between minute ventilation and carbon dioxide output below the ventilatory threshold were calculated by linear regression using the least squares method. Predicted values for maximal oxygen uptake were derived using Jones' equation (Jones, 1988).

2.2.4 Resting hemodynamics

Supine resting right heart hemodynamic measurements were performed in all heart failure patients evaluated for cardiac transplantation. After transplantation supine resting central hemodynamic measurements were repeated at the time of a routine endomyocardial biopsy at 1-2 weeks and at 3 and 12 months post surgery as previously described (Pflugfelder et al., 1988). Briefly, after the endomyocardial biopsy, performed through the right internal jugular vein, a flow directed thermodilution catheter was introduced and advanced into a branch of the right pulmonary artery for resting supine central hemodynamic measurements. Central hemodynamics were measured in the following sequence: pulmonary artery phasic and mean pressures, mean pulmonary capillary wedge pressure, and mean right atrial pressure. Thermodilution cardiac outputs were measured in duplicate or triplicate to obtain values in agreement by 10%.

LIST OF APPENDICES

This thesis is mainly based on the following papers.

I. Bussières-Chafe LM, Pflugfelder PW, Ahmad D, Taylor AW, Weernick C, Kostuk WJ. Cardiopulmonary responses to exercise following cardiac transplantation in patient with early rehabilitation. To be submitted for publication. Med Sci Sport Exerc.

II. Bussières-Chafe LM, Pflugfelder PW, Ahmad D, Taylor AW, Kostuk WJ. Early changes resting pulmonary function following cardiac transplantation. To be submitted for publication. Chest.

III. Bussières-Chafe LM, Pflugfelder PW, Taylor AW, Noble EG, Kostuk WJ. Peripheral skeletal muscle adaptations after cardiac transplantation. To be submitted for publication. Circulation.

IV. Bussières-Chafe LM, Pflugfelder PW, Menkis AH, Novick RJ, McKenzie FN, Taylor AW, Kostuk WJ. Basis for aerobic impairment in patients late post cardiac transplantation. To be submitted for publication.

V. Bussières-Chafe LM, Pflugfelder PW, Ahmad D, Taylor AW, Kostuk WJ. Cardiopulmonary responses to exercise in cardiac transplant recipients are influenced by body position. Submitted for publication. Am J Cardiol.

VI. Bussières-Chafe LM, Pflugfelder PW, Henderson AR, MacKinnon D, Taylor AW, Kostuk WJ. Effect of filling pressure on the release of atrial natriuretic peptide during exercise in cardiac transplant recipients. To be submitted for publication. Can J Cardiol.

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

CHAPTER ONE

GENERAL INTRODUCTION AND BACKGROUND

Few medical therapies can more dramatically improve the quality of life than cardiac transplantation, which is now a well established therapy for end-stage cardiac disease. The first orthotopic human to human heart transplantation was performed on December third 1967, by Christian Barnard in Cape Town, South Africa. This breakthrough was the result of pioneer work done by Shumway and Lower (Griepp and Ergin, 1984) who developed a surgical technique for orthotopic cardiac transplantation, using dogs as a model. Thereafter cardiac transplantation gained some early popularity among a few centres all over the world. However, with the early death of the majority of the recipients because of infection and rejection, the initial enthusiasm waned and most centres abandoned heart transplantation for a decade or so. In the early nineteen eighties, with the introduction of cyclosporine (Calne et al., 1978) and the widespread use of the percutaneous cardiac biopsy technique (Caves et al., 1973) as methods to treat and diagnose rejection, survival after transplantation increased considerably. These interventions, in addition to better cardiac preservation techniques, donor selection, and management strategies, have brought the one year survival rate after transplantation to its current value of greater than 80 % in most centres (Kaye, 1992). This is much better than the grim survival rate of less than 40 % observed in patients considered for transplantation but not operated on (Warner-Stevenson and Miller, 1991). Accordingly, there has been an exponential increase in the number of cardiac transplantation procedures performed (Modry et al., 1986). As more patients

undergo and survive cardiac transplantation, there also has been a change in the research interest. While earlier investigations studied the predictors of survival after transplantation the quality of life and the exercise tolerance following transplantation are now being evaluated.

1.1 EXERCISE CAPACITY AFTER CARDIAC TRANSPLANTATION

Most patients selected for this type of surgery have end stage cardiac failure with New York Heart Association (NYHA) functional classes III to IV disability. However more than 95 % of those who survive 1 year return to NYHA functional class I (Pennock et al., 1982). Preoperatively, the exercise capacity is limited by dyspnea, exhaustion and cachexia. Within days of operation the recipients become ambulatory and within weeks are able to participate in aerobic exercise classes. Some heart transplant recipients have completed mini-triathlons, fun runs and one has even completed the Boston marathon only 15 months after transplantation (Kavanagh et al., 1986).

Many studies have previously described the exercise capacity after cardiac transplantation (Banner et al., 1989; Brubaker et al., 1993; Kavanagh et al., 1988; Labovitz et al., 1989; Marzo et al., 1992; Pope et al., 1980; Savin et al., 1980) however they have compared it to age-matched controls (Brubaker et al., 1993; Kavanagh et al., 1988; Labovitz et al., 1989; Pope et al., 1980; Savin et al., 1980) or coronary artery bypass surgery patients (Degré et al., 1987; Ehrman et al., 1992). Very few studies have been longitudinal (Marzo et al., 1992) and have looked at the exercise response before and after transplantation (Levine et al., 1986; Marzo et al., 1992). The time course and mechanism of the improved exercise capacity after transplantation have not been studied systematically. While improved cardiac function due to the new heart is probably the most important factors for the increased exercise capacity after transplantation little

has been published regarding changes in other organ systems that could also contribute. The recent realization that ventilatory, neurohumoral and peripheral skeletal muscle abnormalities play an important role in limiting the exercise capacity in patients with heart failure (Francis, 1987; Myers et al., 1992), suggests that the decreased exercise capacity in these patients is multisystemic in origin. It is reasonable therefore to assume that the improved exercise capacity after cardiac transplantation could be due to the reversal (partial or complete) of some of those multisystemic adaptations to the chronic cardiac failure state. Recent reports on improved pulmonary function tests (Hosenpud et al., 1990; Ravenscraft et al., 1993), neurohumoral profiles (Olivari et al., 1987) and peripheral vascular compliance (Sinoway et al., 1988) after transplantation, support these assumptions. However, serial changes in resting and exercise lung function and peripheral skeletal muscle adaptations in the early post-operative period after cardiac transplantation have not been described.

The medical community initially responded to heart transplantation surgery with astonishment, when reports first appeared concerning the striking improvement in the exercise capacity of the recipients. However it has now become apparent that exercise capacity is limited in these patients when compared to age-matched controls. Most studies agree that on average cardiac transplant recipients achieve a peak oxygen uptake that is only 2/3 that of normal controls (Brubaker et al., 1993; Kavanagh et al., 1988; Savin et al., 1980). The reason for this decreased peak oxygen uptake is not well understood. The literature has focused on the recipient heart as the central cause of these limitations. After all the recipient's heart is denervated and often develops an accelerated form of coronary artery disease (Uretsky et al., 1987). However other factors could play an important role in limiting exercise capacity such as impaired recovery of the pulmonary function, peripheral vasculature and

musculoskeletal system after transplantation due to irreversible changes from long standing heart failure (Sullivan et al., 1990; Zelis et al., 1982) and/or inactivity prior to transplantation. In addition, immunosuppressive medication (cyclosporine and prednisone) might limit the exercise performance following transplantation.

Cardiac transplant recipients have initially been of great interest to physiologists because of the opportunity to study the denervated heart and ensuing altered cardiac function during exercise. Recent studies have described specific resting and exercise diastolic dysfunction caused not only by denervation (Young et al., 1987) but also by ischemia at the time of harvesting (Burt and Copeland, 1986), myocardial fibrosis (Pickering and Bouginner, 1990), donor size mismatch (Hosenpud et al, 1989b), and rejection. These diastolic dysfunction abnormalities become more apparent in the supine posture (Rudas et al., 1990; Hosenpud et al., 1989a). Marked increases in ventricular filling pressures have been observed, for example, during supine exercise in cardiac transplant recipients, whereas the responses during upright exercise are comparable to normal responses (Rudas et al., 1990). The physiological consequence of this abnormal response during supine exercise has not been well investigated.

1.2 THESIS OUTLINE

The purpose of the present thesis is to investigate the magnitude, time course and cardiopulmonary and peripheral skeletal muscle change associated with the improved exercise capacity following cardiac transplantation. In addition, the present thesis investigates the cardiovascular and pulmonary physiology of the long term cardiac transplant survivor. The thesis is divided into seven chapters (papers), with each chapter providing pertinent background and

literature review. The chapters are arranged in a temporal sequence. The first three chapters relate to the early changes following transplantation whereas, the last four are related to the physiology of the patients late after cardiac transplantation. Due to the limited number of transplant procedures, seven patients were involved simultaneously in the first three studies.

Chapter two investigates the change in the cardiopulmonary response to exercise after transplantation. Chapters Three and Four investigate the resting pulmonary function, and peripheral skeletal muscle changes in the first year following transplantation. While a study of all the changes associated with improved exercise capacity after transplantation is beyond the scope of a single thesis the present results add new knowledge to the current literature.

In Chapter Five, some of the potential mechanisms for the limited peak oxygen uptake of patients late after cardiac transplantation are analyzed.

In view of the marked difference in the hemodynamic responses during supine and upright exercise in cardiac transplant patients, Chapter Six describes the effect of exercise posture on the slope of the cardiac output-oxygen uptake relationship. Similarly Chapter Seven reports the effect of exercise posture, on the peak atrial natriuretic peptide level.

Heart failure is due to a variety of cardiac diseases which includes idiopathic cardiomyopathy, ischemic heart disease, congenital heart disease, viral myocarditis, post partum cardiomyopathy, and valvular heart disease. However end-stage heart failure from either idiopathic cardiomyopathy or ischemic heart disease are the most common pre-operative diagnoses in patients undergoing cardiac transplantation. Chapter Eight describes the exercise response of two patients who underwent cardiac transplantation for a rare cause of end-stage heart failure, mitochondrial myopathy.

CHAPTER TWO

EARLY CHANGES IN THE CARDIOPULMONARY RESPONSE TO EXERCISE AFTER CARDIAC TRANSPLANTATION

2.1 INTRODUCTION

Few interventions for end-stage heart failure can more dramatically improve exercise capacity than cardiac transplantation, now a well established therapy for end stage heart disease (Pennock et al., 1982). In patients with severe cardiac failure, hypoperfusion of working muscle and pulmonary congestion from left ventricular dysfunction lead to early fatigability and exertional dyspnea, which limit exercise capacity (Fowler, 1991; Franciosa et al., 1984). While all patients selected for cardiac transplantation have end-stage cardiac failure with New York Heart Association functional class III or IV disability, more than 95% of those who survive 1 year return to functional class I status (Pennock et al., 1982). The time course and magnitude of the improvement in exercise capacity and the associated changes in the cardiovascular and pulmonary responses to exercise have not been well described.

In the present study, we have investigated the changes in peak oxygen uptake and cardiopulmonary response to exercise in the first 12 months after cardiac transplantation in patients enrolled in an early rehabilitation program.

2.2 METHODS

2.2.1 Patient population

Nineteen patients (one female and 18 males) who underwent successful cardiac transplantation and survived the first post operative year were included in the study. The mean age at the time of transplantation was 46±2 years (range 27-60). All patients were in New York Heart Association functional class III to IV prior to transplantation. The etiology of end-stage heart failure was coronary artery disease in eight patients, cardiomyopathy in 10 and valvular heart disease in one patient. While awaiting cardiac transplantation, all patients were treated with an optimal anti-failure regimen, which included diuretics, angiotensin converting enzyme inhibitors, and digoxin. After transplantation, the immunosuppressive regimen consisted of prednisone and cyclosporine to which azathioprine was added within one month in three patients, within three months in five, and within 12 months in seven. The average dose of prednisone was 20±1 mg, 18±1 mg and 7±1 mg at one, three and 12 months respectively. While the average daily dose of cyclosporine was 600 mg, 450 mg and 350 mg respectively. Other medications in the post operative period included diuretics (in seven patients at one month, four at three and four at 12 months), calcium channel blockers (in two patients at one month, four at three months, and eight at 12 months) and angiotensin converting enzyme inhibitors (in one patient at one and three months, and two patients at 12 months).

2.2.2 Rehabilitation program

At our centre all cardiac transplant patients are enrolled in a physical rehabilitation program in the first three months after transplantation. All patients in the present study participated in the program. The program begins with rapid mobilization and bed exercises in the first operative week. Thereafter and up to three months a more structured exercise program (five mornings/week) is

initiated. The exercise regimen consists of 60 minutes of supervised stationary cycling, treadmill exercise, stair climbing, upper and lower extremity strengthening as well as stretching exercises. Exercise duration and intensity of the cycle and treadmill exercise increases with patient recovery. On average patients cycle or walk on the treadmill on alternating days at each session for 10 to 30 minutes. The intensity is designed to elicit perceive exertion rating (modified Borg scale) of 4-6 (somewhat hard). After three months, there was no organized rehabilitation program. While patients were encouraged to maintain fitness and received instructions and personal recommendations to continue exercise training at home, only three patients exercised regularly for 30 min at least 3 time a week at 12 months post transplantation.

2.2.3 Cardiopulmonary exercise testing

Symptom-limited maximal upright graded exercise testing was performed on an electronically-braked cycle ergometer. Prior to transplantation seven of the 19 patients were well enough to undergo an exercise test after being accepted for cardiac transplantation. One patient did not undergo exercise testing at one and three months because of pneumocystis carnii pneumonia, while another patient was not exercised at one month because of a leaking lymphocele in the femoral area a complication from an intra aortic balloon at the time of transplantation. The initial workload was 12.5 or 25 Watts depending on the patient's ability to exercise. The workload increased by 12.5 or 25 Watts every three minutes until fatigue. Blood pressure was taken by sphygmomanometer at rest, while sitting on the cycle ergometer, and in the final minute of each exercise stage. Mixed expired gases were analyzed continuously during the exercise test. Subjects breathed through a low resistance, high velocity valve (Hans Rudolph) connected on the inspired side to a gas meter and on the expired side to a 4.5 litre mixing chamber. Expired

gases were sampled continuously (rate 4-6 L/min) and analyzed for O_2 and CO_2 content (Ametek). Data were processed on line by a microcomputer programmed to correct for delays between the signals for volume and gas concentration. Average values for each 15 seconds interval were presented for the following variables: respiratory rate, minute ventilation, tidal volume, oxygen uptake, carbon dioxide output, and heart rate. The anaerobic threshold was determined non-invasively using the V-slope method (Beaver et al., 1986) with the mathematical calculation performed by the computer. Individual slopes of the relationship between minute ventilation and carbon dioxide output below the ventilatory threshold were calculated by linear regression using the least squares method. Predicted values for maximal oxygen uptake were derived using Jones' equation (Jones, 1988).

2.2.4 Resting hemodynamics

Supine resting right heart hemodynamic measurements were performed in all heart failure patients evaluated for cardiac transplantation. After transplantation supine resting central hemodynamic measurements were repeated at the time of a routine endomyocardial biopsy at 1-2 weeks and at 3 and 12 months post surgery as previously described (Pflugfelder et al., 1988). Briefly, after the endomyocardial biopsy, performed through the right internal jugular vein, a flow directed thermodilution catheter was introduced and advanced into a branch of the right pulmonary artery for resting supine central hemodynamic measurements. Central hemodynamics were measured in the following sequence: pulmonary artery phasic and mean pressures, mean pulmonary capillary wedge pressure, and mean right atrial pressure. Thermodilution cardiac outputs were measured in duplicate or triplicate to obtain values in agreement by 10%.

2.2.5 Statistical analyses

Values are presented as means ± SEM. Pre and post transplantation comparisons of resting hemodynamics were made analysis of variance with repeated measures. Because of the limited number of patients tested prior to transplantation their values were compared to the after transplantation values using a oneway analysis of variance, while the exercise test variables measured at 1, 3 and 12 months were compared using a repeated measures oneway analysis of variance. To determine the differences between the ventilatory response to carbon dioxide output, the slope of the regression lines were compared. Statistical significance was determined by a p value <0.05.

2.3 RESULTS

The supine resting right atrial and pulmonary capillary wedge pressures decreased and the cardiac index increased significantly in the early period after transplantation and thereafter remained unchanged (Table 1).

In the seven patients well enough to undergo an exercise test prior to transplantation the peak oxygen uptake was markedly reduced, on average to 29% of predicted values (Table 2). Peak oxygen uptake expressed as ml/kg/min increased significantly in the first three months after cardiac transplantation but did not increase further by 12 months. The ventilatory threshold also increased with time post-transplantation but the increased was of less magnitude so that the relative % VO_2 at the ventilatory threshold decreased early after transplantation (Table 2).

The resting heart rate in patients before and after transplantation (Table 2). With exercise there was a significant increase in heart rate in all groups. However, a significant higher heart rate occurred at peak exercise after transplantation despite cardiac denervation (Table 2).

Resting systolic and diastolic blood pressures in the patients before transplantation were low (Table 2). Resting systolic and diastolic blood pressures increased significantly in the first three months after transplantation (Table 2). During exercise, patients before transplantation had a blunted blood pressure response to exercise, after transplantation patients had an average increased (peak-rest) in systolic blood pressure of 27±3 mmHg at one month, 28±4 mmHg at three months and 30±5 mmHg 12 months. In all groups, diastolic blood pressure did not change significantly with exercise. Figure 1 illustrated the increased in blood pressure normalized for work in the seven patients tested before and after transplantation.

The resting values for minute ventilation, tidal volume, and respiratory rate did not change after transplantation. With exercise, minute ventilation increased significantly at all times. However, at peak exercise, minute ventilation was higher following transplantation. Because the peak respiratory rate was similar at all times, the improved minute ventilation after transplantation was achieved by an augmented peak tidal volume (Table 2, Figure 2). Despite lower minute ventilation at peak exercise, patients before transplantation had a significantly higher minute ventilation for a given level of carbon dioxide output. The slope of the ventilation to carbon dioxide output (V_E/V_{CO_2}) relationship decreased significantly by three months after transplantation and a further decrease was noted by 12 months. Figure 3 shows the effect of transplantation on the relationship of the ventilation to carbon dioxide output (V_E/V_{CO_2}) in the seven patients tested before and after transplantation.

Table 1. Resting supine hemodynamics before and at one to two weeks and at three, and 12 months following cardiac transplantation

	Pre n=19	1-2 wks n=18	3 mo n=19	12 mo n=19
RAP (mmHg)	13±2	8±1*	6±1*	5±1*
PCWP (mmHg)	23±2	14±1*	12±1*	13±2*
CI (L/min/m^2)	1.9±0.1	3.0±0.1*	3.0±0.1*	3.3±0.1*

Means ± SEM; CI=cardiac index; PCWP=pulmonary capillary wedge pressure; RAP=right atrial pressure

* $p<0.05$ vs Pre

Table 2. Cardiopulmonary exercise test results before and at one, three, and 12 months following cardiac transplantation

	Pre n=7	1 mo n=17	3 mo n=18	12 mo n=19
Weight (kg)n=18	71±3	69±2	71±2	78±3**
VO_2 max (ml/kg/min)	9.8±0.5	16.8±1.2*	19.9±1.3**	20.2±1.5**
VO_2 max (% predicted)	29±1	49±3*	58±3**	59±4**
VT (ml/kg/min)	7.5±0.6	10.0±0.4*	11.4±0.4**	11.8±0.7**
VT/VO_2 max (%)	76±2	60±2*	59±2*	58±2*
HR rest (beats/min)	80±8	94±2	96±3	93±3
HR max (beats/min)	101±8	117±4	128±4**	132±3**
BP sys rest (mmHg)	89±6	124±4*	150±5**	149±4**
BP sys max (mmHg)	94±7	151±5*	177±5**	179±6**
BP dias rest (mmHg)	63±5	77±2*	96±3**	96±2**
BP dias max (mmHg)	65±4	77±2	95±4**	96±4**
VE max (L/min)	33.5±2.4	57.3±4.2*	70.4±5.5**	63.3±4.6*†
TV max (L)	1.27±0.06	1.98±0.16	2.38±0.16**	2.34±0.16**
RR max (RRM)	27±2	29±1	30±1	27±1
VE/VCO_2 (L/min)	45.1±2.5	38.6±1.9	35.4±1.5*	31.4±1.6***
RER	1.02±0.02	1.19±0.11*	1.22±0.09*	1.16±0.02*†

Means ± SEM; BP sys=systolic blood pressure; BP dias=diastolic blood pressure; HR=heart rate; RR=respiratory rate; RRM=respiratory rate per minute; TV=tidal volume; VE=minute ventilation; VE/VCO_2=relation between minute ventilation and carbon dioxide output; VO_2 max=peak oxygen uptake; VT=ventilatory threshold.

* $p<0.05$ vs Pre; ** $p<0.05$ vs Pre and one month; *** $p<0.05$ vs pre, and one and three months; † $p<0.05$ vs 3 months

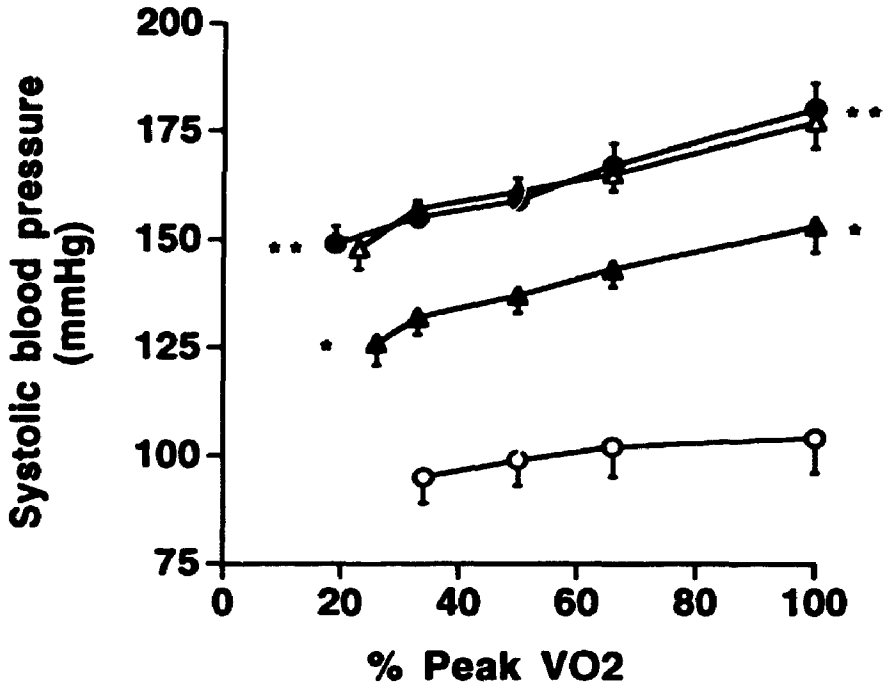


Figure 1. Blood pressure response as a function of normalized work (% peak oxygen uptake) in seven patients tested before (open circle) and at one (closed triangle), three (open triangle) and 12 months (closed circle) following cardiac transplantation. Values are means±SEM. * Significantly different from pre transplant value ($p<0.05$); ** Significantly different from pre and one month post transplantation ($p<0.05$).

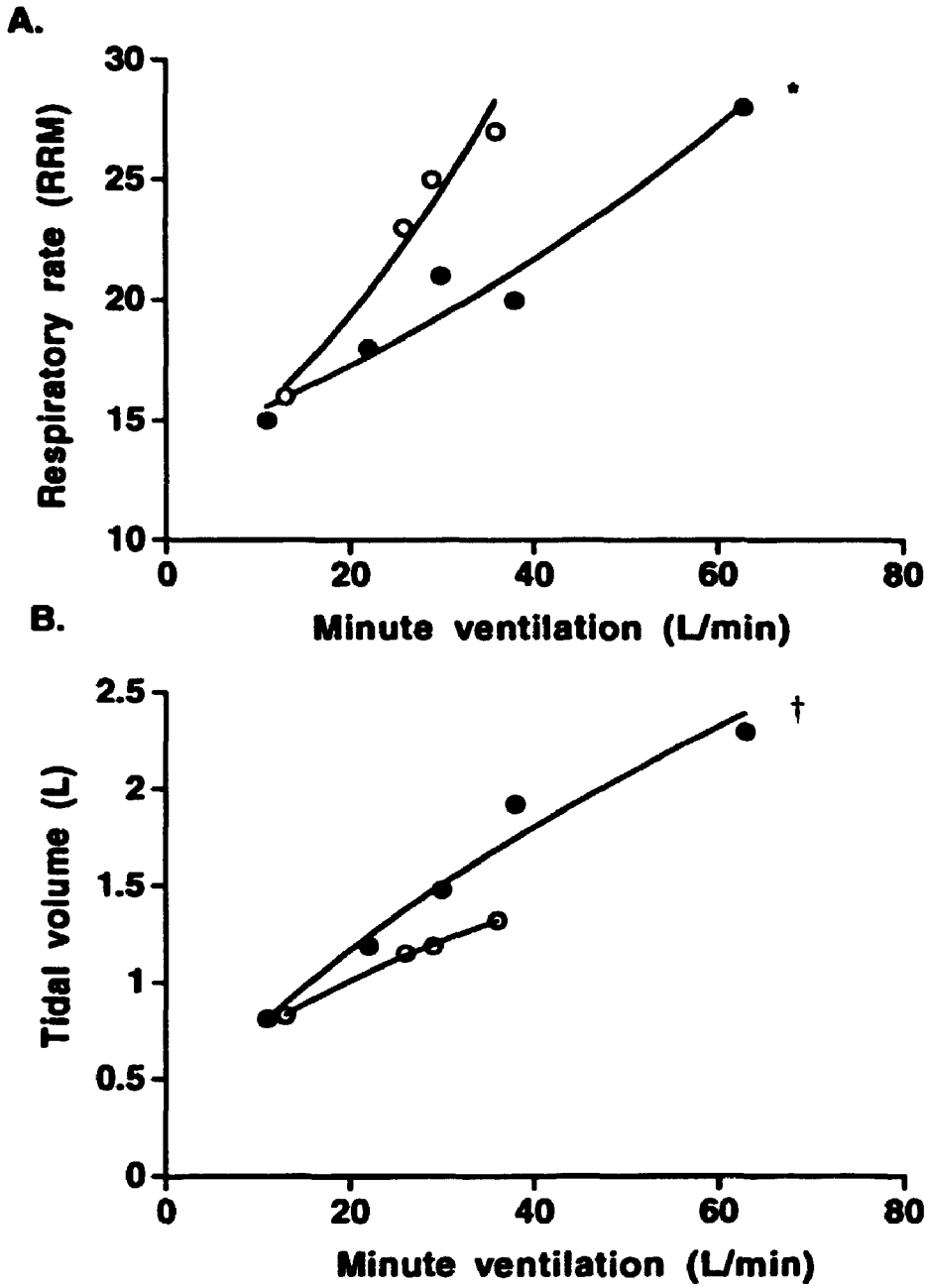


Figure 2. The relationship between minute ventilation, respiratory rate (A) and tidal volume (B) in 7 patients tested before (open circle) and at 12 months (closed circle) following cardiac transplantation. * Peak minute ventilation significantly different from pre transplantation ($p<0.05$); † Peak tidal volume significantly different pre transplantation ($p<0.05$).

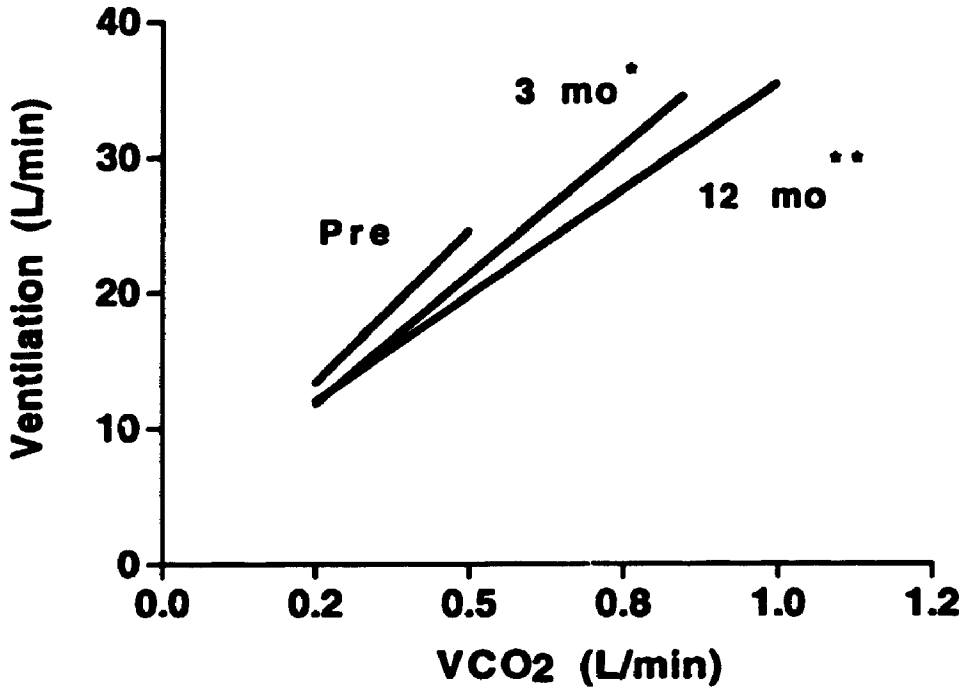


Figure 3. The relationship of minute ventilation and carbon dioxide output below the ventilatory threshold before and at three and 12 months following cardiac transplantation in seven patients tested before and after transplantation. * Significantly different from pre ($p<0.05$); ** Significantly different from pre and three months ($p<0.05$).n

2.4 DISCUSSION

Patients with heart failure assess for cardiac transplantation have a marked decrease in exercise capacity (Mancini et al., 1991). The present study provides some insight into the time course and magnitude of the changes in exercise capacity after cardiac transplantation in patients enrolled in a rehabilitation program in the first three months after transplantation. Increased peak oxygen uptake was observed as early as one month after transplantation and improved further by three months. The increased peak oxygen uptake was associated with improved resting hemodynamics and peak systolic blood pressure, in addition to increased peak minute ventilation and decreased ventilatory response to exercise.

At our centre, a rehabilitation program is initiated within days of the transplantation and continued for 3 months in all patients. Since it was not possible to organize a matched-control series, it is difficult from the present results to dissociate the contribution of the new heart and the exercise program in the improvement in exercise capacity early after transplantation. Previously published report on cardiac rehabilitation program in patients with heart failure (Coats et al., 1992; Sullivan et al., 1988) and cardiac transplant recipients (Kavanagh et al., 1988; Keteyian et al., 1990; Niset et al., 1988) have observed a 15 to 33 % increase in peak oxygen uptake. The marked increase in peak oxygen uptake (≈100%) at 3 months post transplantation, when compared to before transplantation, suggests that the new heart play an important role in the improved exercise capacity after transplantation. However, because the 3 months peak oxygen uptake (at the end of the rehabilitation program) was similar to the value obtained at 12 months and to the reported value of long term cardiac transplant recipients (Banner et al., 1989; Kavanagh et al., 1988; Savin et al., 1980), we concluded that the rehabilitation program, may have

accelerated the rate of recovery. The finding that our three months value is higher than those reported by Ehrman et al. (1992) support this hypothesis.

Despite marked improvement in exercise capacity after transplantation in the present study the peak oxygen uptake at 12 months post transplantation corresponded to only 59 % of predicted value. This limited exercise capacity have been observed by others in late cardiac transplant recipients (Bussières-Chafe, et al., 1993; Kavanagh et al., 1988; Savin et al., 1980). It is possible that if the supervised rehabilitation program had been extended to beyond three months, a higher peak oxygen uptake might have been observed late post-transplantation. Kavanagh et al. (1988) have reported peak oxygen uptakes approaching normal values in cardiac transplant recipients after a two year walk/jog intensive exercise program. However, whereas prolonging the rehabilitation program could have increased the peak oxygen uptake in some patients, in many, the long term rehabilitation potential may be limited due to the development of post operative complications (Labovitz et al., 1989; Hosenpud et al., 1989a), the use of medications (Scherrer et al., 1990), and possibly irreversible changes from long standing heart failure and inactivity (Bussières-Chafe et al., 1993; Savin et al., 1980).

Since peak oxygen uptake is influenced by patient motivation, anaerobic threshold, an objective index of aerobic capacity not influenced by motivation was measured non-invasively. The ventilatory threshold is reproducible even in heart failure patients, (Itoh et al., 1990; Weber and Janicki, 1985) and heart transplant recipients (Brubaker et al., 1993). We have observed an increase in the ventilatory threshold after transplantation, confirming that the higher exercise capacity was the result of physiological changes leading to improved oxygen delivery and utilization. However the magnitude of the increase in ventilatory threshold was less than the peak oxygen uptake so that the relative

% VO_2 at the ventilatory threshold decreased after transplantation (Table 2). Prior to transplantation patients had a high relative % VO_2 at the ventilatory threshold (76±2 %). Itoh et al. (1990) have observed an increase in the relative % VO_2 at the ventilatory threshold in patients with severe heart failure. The early decrease in the relative % VO_2 at the ventilatory threshold coupled with the higher peak RER after transplantation suggest that patients were able to exercise at a higher intensity. This is possibly the result of decrease pulmonary congestion or increased peripheral blood flow. The persistence of a low relative % VO_2 at the ventilatory threshold at three and 12 months post transplantation suggests that, unlike the well trained athlete (Hurley et al., 1984), transplant patients might have minimal peripheral adaptations. This limited peripheral adaptations could be due to irreversible changes from prolong period of inactivity or changes due to prednisone and cyclosporine treatment. Brubaker et al. (1993) have observed similar relative % VO_2 at the ventilatory threshold in cardiac transplant recipients and have confirmed this values using lactate measurements.

One of the limitation in the present study is that only about half of the patients exercised prior to transplantation. We believe however that the cardiopulmonary response to exercise in the remaining patients is representative, and may even overestimate the degree of response of the non-exercised patients, since most of them could not be exercised because of the severity of their condition.

During exercise, patients with congestive heart failure have an attenuated heart rate and blood pressure response compared to healthy control subjects (Weber and Janicki, 1985; Higginbotham et al., 1983). In part, this is the result of a diminished sympathetic nervous system response to exercise (Francis et al., 1985) and reduced density of ß-adrenergic receptors in the

failing myocardium (Bristow et al., 1982). In the present study patients before transplantation exhibited blunted heart rate and systolic blood pressure responses to exercise. As a result of cardiac denervation, cardiac transplant, recipients have also an atypical heart rate response to exercise. The response is biphasic (Kavanagh et al., 1988; Pickering et al., 1989) and lower in amplitude (Quigg et al., 1989) than that seen in normal subjects. During early exercise, a small increase in heart rate occurs and may be due to intrinsic myocardial heart rate regulation. During later stages of exercise, heart rate increases at a more rapid rate as a result of increasing levels of circulating catecholamines (Popes et al., 1980). The rate of increase, however, is less than normal because there is no direct sympathetic stimulation (Quigg et al., 1989). Despite denervation after transplantation the peak heart rate was significantly higher than pre-transplant values by 3 months post transplantation but remained much below the age-adjusted maximal heart rate of 174 beats/min.

A marked increase in the resting and peak systolic and diastolic blood pressure was also observed early after cardiac transplantation with no further improvement late post-transplantation. The increase in resting and peak blood pressure in the first month post transplantation is due to the improved cardiac function thereafter the resting blood pressure continue to increase possibly due to the hypertensive effect of cyclosporine therapy (Myers et al., 1984). Antihypertensive therapy, instituted in more than 70% of patients at 12 months post-transplantation, may have significantly influenced blood pressure response to exercise.

A reduction in static lung volume has been observed in patients awaiting cardiac transplantation (Wright et al., 1990; Hosenpud et al., 1990; Bussières et al., 1990) and has been attributed to cardiac enlargement, pleural effusions, and pulmonary edema. With normalization of cardiovascular physiology after

cardiac transplantation, substantial improvement in resting lung function has been documented (Hosenpud et al., 1990; Bussières et al., 1992; Ravenscraft et al., 1993). During dynamic exercise, Weber et al. (1982) have reported lower peak minute ventilation and a restrictive pattern of ventilation in patients with severe congestive heart failure when compared to less impaired patients or normals. The present study is in concordance with those findings and further illustrates that dynamic lung function abnormalities are also in part reversible after cardiac transplantation. During submaximal exercise, patients after transplantation had larger tidal volumes and lower respiratory rates for a given minute ventilation (Figure 2). At peak exercise, minute ventilation was higher after transplantation due to an increased tidal volume. The increased tidal volume at peak exercise is possibly the result of increased lung compliance and blood supply to respiratory muscles during exercise brought about by improved cardiac function.

Despite having lower minute ventilation and tidal volume at peak exercise, patients with congestive heart failure have an augmented ventilatory response during exercise when compared to normal subjects (Buller et Poole-Wilson, 1990; Gazetopoulos et al., 1966; Sullivan et al., 1988; Weber et al., 1982; Wilson and Ferraro , 1983). The pathophysiologic basis for this increase in ventilatory response to carbon dioxide output is not fully understood. Numerous underlying mechanisms have been suggested including, early onset of systemic and skeletal muscle acidosis (Franciosa et al., 1984; Weber et al., 1982), increased dead space to tidal volume ratio from lung congestion and changes in lung compliance (Clark et al., 1992; Sullivan et al., 1988), increased pulmonary capillary wedge pressure (Gazetopoulos et al., 1966), and increased ventilation perfusion mismatch (Buller and Poole-Wilson, 1990; Myers et al., 1992; Sullivan et al., 1988; Wada et al., 1993). In the present study, patients,

prior to transplantation, had a markedly elevated minute ventilation to carbon dioxide ratio in response to submaximal exercise. The slope of the relationship was significantly lower early after transplantation and continued to decrease by 12 months. These changes are likely mediated by improved lung compliance and ventilation perfusion matching. It is possible that improved peripheral skeletal muscle perfusion, after transplantation could also have contribute to the observed decrease in the slope of the minute ventilation to carbon dioxide output during exercise due to decrease ergoreceptor activation (Piepoli et al., 1993). Despite the marked decrease in the slope of the minute ventilation-carbon dioxide output relationship after transplantation, the slope did not return to the reported normal value of around 23 (Rebuck et al., 1972), which is in agreement with Degré et al. (1987) and Marzo et al. (1992) but in contrast to Theodore et al. (1986). Cyclosporine effects on the pulmonary vasculature, coexisting pulmonary disease from previous smoking and/or permanent lung changes from long standing pulmonary venous hypertension may all have contributed to this finding.

In summary, improved exercise performance after cardiac transplantation is associated with changes in both respiratory and cardiovascular physiology. The increased peak oxygen uptake after cardiac transplantation is due to the new heart however a rehabilitation program in the first three months post cardiac transplantation may accelerate the rate of recovery in these patients since, in the present study, the peak oxygen uptake at three months post cardiac transplantation (at the termination of the rehabilitation program) was similar to the peak oxygen uptake at 12 months and to the reported values for patients more than one year after cardiac transplantation.

CHAPTER THREE

EARLY CHANGES IN STATIC LUNG FUNCTION AFTER TRANSPLANTATION

3.1 INTRODUCTION

Restrictive ventilatory defects and decreased diffusing capacity (DLco) have been described at rest in patients with chronic heart failure (Gray et al., 1979; Collins et al., 1975; Ries et al., 1986; Smulyan et al., 1974). During exercise, rapid shallow breathing is characteristic (Weber et al., 1982), together with increased ventilation for a given metabolic demand e.g. carbon dioxide output (Wilson and Ferraro, 1983). These changes are related to the severity of heart failure and contribute to decreased exercise tolerance (Weber et al., 1982; Wilson and Ferraro, 1983; Myers et al., 1992; Buller and Poole-Wilson,1990).

In patients with end-stage heart failure awaiting cardiac transplantation pulmonary function abnormalities have also been described (Bussières et al., 1990; Casan et al., 1987; Hosenpud et al., 1990; Ohar et al., 1993; Ravenscraft et al., 1993; Wright et al., 1990). Cardiac transplantation provides a unique opportunity for evaluating the effect of improved cardiac performance on pulmonary function. While studies have documented normalization of right and left filling pressures within the first few weeks post transplantation (Bhatia et al., 1987), most resting pulmonary function tests have been reported in late cardiac transplant recipients only (Hosenpud et al., 1990; Casan et al., 1987; Ravenscraft et al., 1993; Ohar et al., 1993). There has been no report of serial resting pulmonary function in the early period post transplantation. In the present

investigation we have measured the pulmonary function tests before and serially in the first 12 months after cardiac transplantation.

3.2 METHODS

3.2.1 Patient population

Fourteen male patients were studied prior to and following cardiac transplantation. Their mean age at the time of transplantation was 47±3 years (range 23 to 58). The mean height was 1.73±0.02 cm and their weight at the time of transplantation was 76±3 kg. The weight was 72±3 kg, 73±3 kg, and 84±3 kg at one , three and 12 months post transplantation respectively (12 months $p<0.05$ vs 1 and 3 months). The etiology of heart disease leading to transplantation was coronary artery disease in six patients, cardiomyopathy in seven and valvular heart disease in one. Ten patients had smoked prior to transplantation, however six had stopped at least two years before the transplantation. No patients had severe chronic obstructive lung disease prior to transplantation or received any bronchodilator agent during the study period.

The immunosuppressive regimen consisted of prednisone and cyclosporine in all patients. The dose of cyclosporine was on average 600, 400 and 300 mg per day at one, three and 12 months respectively. Azathioprine was added in five patients at one month, six at three months and seven at 12 months post transplantation.

3.2.2 Pulmonary function tests

Pulmonary function test were measured on average 60 days before transplantation (10-380 days). Pulmonary function was repeated at one, three and 12 months after transplantation. Pulmonary function testing included measurement of forced expired volume, lung volume and diffusing capacity. All pulmonary function test results, except FEV_1/FVC ratio and the peak flow rate, are

expressed as a percentage of predicted, based on age, height, weight and sex. Forced expiratory volume at 1 second (FEV_1) and forced vital capacity (FVC) were obtained by spirometry (dry rolling seal) and evaluated utilizing the predicted values of Knudson et al (1983). The predicted values of Goldman et al. (1959) were used for helium dilution determination of lung volume which included total lung capacity (TLC) and residual volume (RV). Diffusing capacity (DLco) was determined using the single breath carbon monoxide technique and values were corrected for hemoglobin concentration (Cotes et al., 1972). The predicted values for the DLco were those of Miller et al (1983).

3.2.3 Resting hemodynamics

Resting supine hemodynamics were measured, after a routine endomyocardial biopsy performed through the right internal jugular vein, using a flow directed balloon-tipped catheter was advanced into a branch of the right pulmonary artery. Filling pressures were measured in a standard manner and were zero referenced to the level of the right atrium as previously described (Pflugfelder et al., 1988). Central hemodynamics were measured in the following sequence: pulmonary artery phasic and mean pressures, mean pulmonary capillary wedge pressure, and mean right atrial pressure. Thermodilution cardiac outputs were measured in duplicate or triplicate to obtain values in agreement by 10%.

3.2.4 Statistical analyses

Descriptive data are presented as means ± SEM. Data analysis was performed using a repeated measures oneway analysis of variance. Correlations between diffusing capacity at 12 months and pre-operative and post-operative measurements was determined using Pearson's correlation coefficient (r). A p value of < 0.05 was considered significant.

3.3 RESULTS

Resting central hemodynamics. Table 3 lists the pre and postoperative resting central hemodynamics. Right and left atrial pressures and cardiac output returned to the normal range within the one to two weeks after cardiac transplantation.

Pulmonary function test. Prior to transplantation, pulmonary function test results were abnormal in many patients. Diffusing capacity was low and abnormal (<75% of predicted when corrected for hemoglobin) in 13 of the 14 patients. A mild restrictive defect (TLC <75% of predicted) was also observed in three of the 14 patients. Only two patients had significant airway obstruction (FEV_1/FVC <70%). After transplantation, there was an increase in forced expired volume. The FEV_1 and FVC increased significantly by 3 months post transplantation and increased further by 12 months (Table 4). Similarly the peak expiratory flow rate increased after transplantation. The increased was however significant only by 12 months post transplantation. Total lung volume also increased significantly by 12 months post transplantation (Table 4) due to increased inspiratory capacity (Figure 4).

Before transplantation patients had low DLco (Table 4). Despite general improvement in lung function, the DLco decreased early post transplantation and returned to within pre transplant values at 12 months. Both the DLco and the Kco (DLco corrected for alveolar volume) remained well below predicted values at 12 months post transplantation (Table 4).

Patients who smoked prior transplantation did not have lower DLco at 12 months. DLco measured at 12 months did not correlate with any pre-transplant or 12 months central hemodynamic measurements or daily dose of cyclosporine at 12 months (Figure 5). However a correlation was observed between pre and 12 months DLco values ($r=0.71$, $p<0.01$, Figure 6).

Table 3. Resting central hemodynamics before, and at one to two weeks, and three and 12 months following cardiac transplantation

	Pre n=14	1-2 weeks n=14	3 mo n=14	12 mo n=14
RAP (mmHg)	14±2	9±1*	7±1*	5±1*
PAP mean (mmHg)	41±3	27±2*	29±3*	24±3*
PCWP (mmHg)	29±2	18±2*	14±1*	13±2*
CI (L/min/ m^2)	1.7±0.1	2.9±0.1*	3.2±0.2*	3.1±0.2*
PVR (Wood units)	3.85±0.70	1.69±0.22*	2.22±0.29*	1.81±0.31*

Means ± SEM; CI=cardiac index; PAPmean=mean pulmonary artery pressure; PCWP=mean pulmonary capillary wedge pressure; PVR=pulmonary vascular resistance; RAP=mean right atrial pressure.

*$p<0.05$ versus Pre

Table 4. Resting pulmonary function tests before and at one, three and 12 months following cardiac transplantation

	Pre n=14	1 mo n=14	3 mo n=14	12 mo n=14
FEV_1(L)	2.83±0.14	2.90±0.19	3.28±0.17**	3.69±0.24**
FEV_1 (% predicted)	80±4	81±5	92±4**	105±6**
FVC (L)	3.73±0.20	3.65±0.20	4.09±0.16	4.87±0.24***
FVC (% predicted)	85±4	83±5	94±4	112±5***
FEV_1/FVC (%)	77±2	79±2	80±2	75±3†
PEFR (L/min)	8.17±0.4	8.20±0.5	9.10±0.31	9.88±.53***
TLC (L)	5.38±0.30	5.42±0.32	5.93±0.20	6.54±0.25***
TLC (% predicted)	80±4	80±4	87±3	97±3***
RV (L)	1.57±0.13	1.67±0.05	1.60±0.10	1.57±0.11
RV (% predicted)	76±4	82±3	77±3	75±5
DLco (ml/min/mmHg)	18.4±1.4	15.2±0.9*	14.2±1.2*	20.0±1.2††
DLco (% predicted)	61±4	51±3*	48±4*	68±3††
Kco (ml/min/mmHg/L)	3.23±0.22	2.66±0.16	2.31±0.22*	3.18±0.15
Kco (% predicted)	57±4	45±3	41±4*	56±2

Means± SEM; DLco=lung diffusing capacity for carbon monoxide corrected for hemoglobin; FEV_1=forced expired volume in the first second; FVC=forced vital capacity; Kco=diffusing capacity corrected for alveolar volume; PEFR=peak expired flow rate; RV=residual volume; TLC=total lung capacity.

*p<0.05 versus Pre; **=p<0.05 versus Pre and one month; ***p<0.05 vs Pre, one and three months; † p<0.05 vs three months; †† p<0.05 vs one and three months

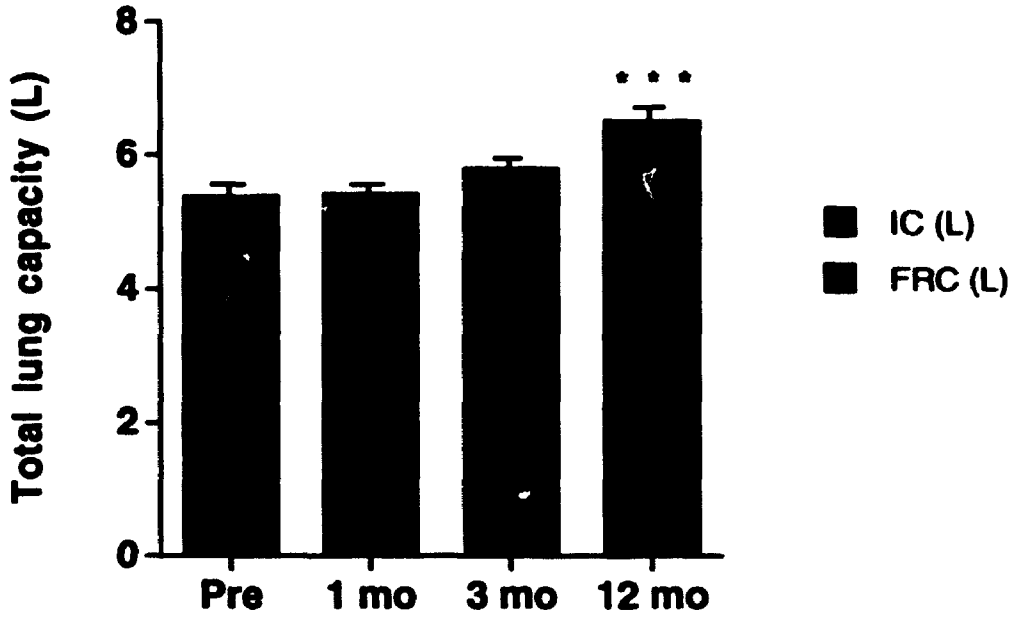


Figure 4. Lung volumes before and at one, three and 12 months after transplantation. Values are means±SEM. * Total lung volume (TLC) and inspiratory capacity (IC) significantly different from pre, one and three months post transplantation ($p<0.05$). FRC=functional residual capacity.

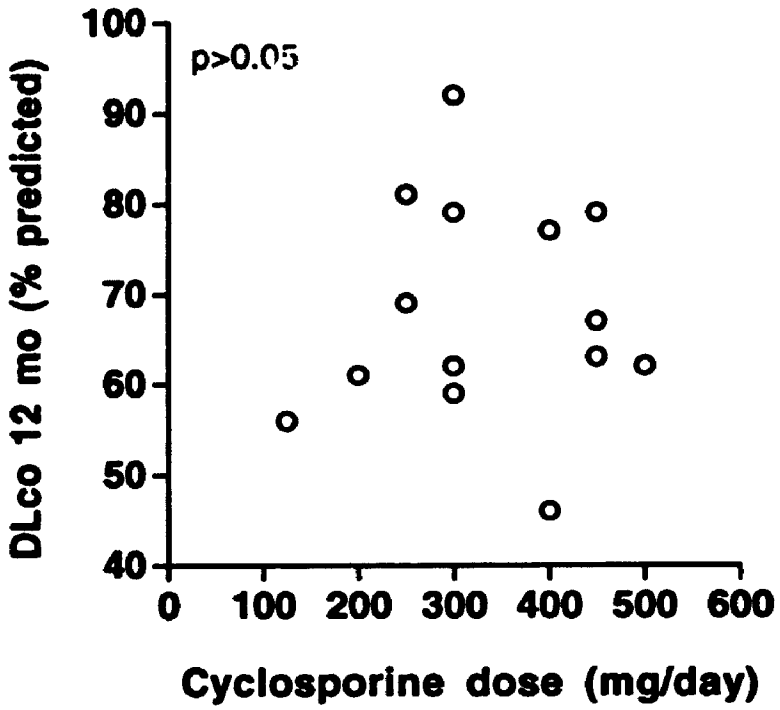


Figure 5. Correlation between diffusing capacity and daily dose of cyclosporine at 12 months after cardiac transplantation

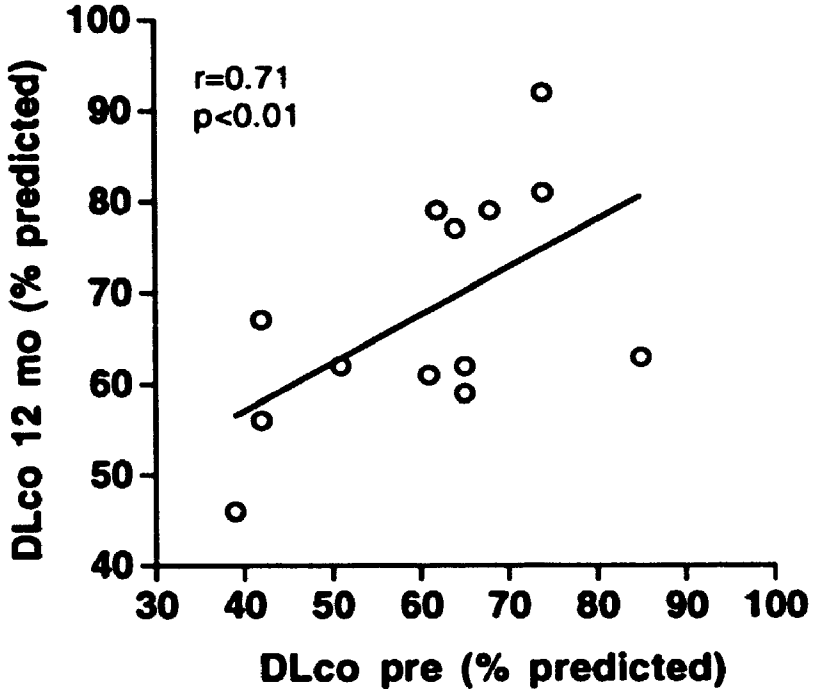


Figure 6. Correlation between diffusing capacity before and at 12 months after cardiac transplantation

3.4 DISCUSSION

Patients awaiting cardiac transplantation have abnormal resting pulmonary function. Although not usually severe, as it is a relative contraindication to cardiac transplantation, some reduction in FVC, FEV_1, TLC and DLco has been described (Bussières et al., 1990; Casan et al., 1987; Hosenpud et al., 1990; Ohar et al., 1993; Ravenscraft et al., 1993; Wright et al., 1990). These abnormalities are due mostly to reduced cardiac function leading to increased cardiac size, pleural effusions and pulmonary congestion. Accordingly, with normalization of cardiac size and central hemodynamics after cardiac transplantation, pulmonary function test abnormalities have been reported to improve (Casan et al., 1987; Hosenpud et al., 1990; Ohar et al., 1993; Ravenscraft et al., 1993). The present results are in agreement with these findings. Furthermore they provide a unique insight into the time course of the early changes in pulmonary function after cardiac transplantation. Some improvements in static lung function were noted as early as in the first three months post transplantation with further improvements by 12 months. Such serial observations have allowed us to evaluate the temporal relationship between improved central hemodynamics and lung function. Although normalization of central hemodynamics occurs early after transplantation, lung function continued to improve up to 12 months post surgery. This delay between the improvement in central hemodynamics and increased lung volumes could be due to the effect of open heart surgery and the slow reversal of pulmonary changes from long-standing pulmonary congestion. A significant decrease in lung compliance and volumes has been reported after coronary artery bypass surgery (Braun et al., 1978; Ellison et al., 1967) and persists up to three to four months post surgery. Similarly, although the greatest improvement in pulmonary function after an acute episode of heart failure occurs within the first few days it

may take months for the pulmonary function to return to normal in some patients (Light and George, 1983). Despite this delay, the recovery of the pulmonary function appears to be satisfactory with mean FEV_1, FVC and TLC values well within the normal range by 12 months post transplantation. The increased inspiratory capacity after transplantation is possibly the result of increased lung compliance from the relief of pulmonary congestion. However the increased peak expiratory flow rate observed at 12 months after transplantation suggest that increased inspiratory capacity could also be due to increased patients effort possibly from increased respiratory muscle strength. Increased peak flow rate have not been previously reported after cardiac transplantation

Despite improvement in lung volume, like others (Casan et al., 1987; Groen et al., 1992; Hosenpud et al., 1990; Kuchipudi et al., 1990; Ohar et al., 1993; Ravenscraft et al., 1993; Wright et al., 1990) we have observed a persistently low DLco after cardiac transplantation. The present study is unique in that we have looked at the early changes in DLco after transplantation. The DLco decreased from preoperative values in first month post transplantation but returned to pre transplant values by 12 months. Even when corrected for alveolar volume (Kco) the diffusing capacity unlike lung volumes remained well below the predicted value. The early decreased in DLco is possibly the result of decreased pulmonary capillary blood volume and the effect of the extracorporeal circulation. Reduced values for DLco have been reported early after coronary artery bypass surgery and these persist up to four months post surgery (Braun et al., 1978). These changes are in part the result of pulmonary vascular injury from lung ischemia and possibly the release of endotoxin and tumor necrosis factor (Dauber et al., 1993) during the bypass. The DLco could remain low thereafter, well below predicted values, due to irreversible changes from chronic pulmonary edema, interstitial damage from subclinical respiratory infections in these

immunocompromized patients and possibly as a result of the gradual effect of cyclosporine toxicity as previously reported (Casan et al., 1987; Groen et al., 1992). The correlation observed between pre and 12 months DLco values suggests an important contribution of the vascular changes from long standing pulmonary congestion prior to transplantation.

In the present study marked improvement in static lung function was observed within the first three months after cardiac transplantation. The changes however lagged behind the improved resting central hemodynamics suggesting a slow recovery of the pulmonary system after prolong period of pulmonary congestion. Increased forced expired volume and total lung volume after transplantation are possibly mediated by improved lung compliance, respiratory muscle strength and patients effort. Unlike the other pulmonary function tests the DLco did not improve after transplantation even when corrected for alveolar volume possibly due to irreversible pulmonary vascular changes from prolong period of pulmonary congestion prior to transplantation.

CHAPTER FOUR

PERIPHERAL SKELETAL MUSCLE ADAPTATIONS AFTER CARDIAC TRANSPLANTATION

4.1 INTRODUCTION

Exercise intolerance in patients with chronic heart failure is complex in origin (Goodman, 1990), involving neurohumoral (Francis, 1987), pulmonary (Myers et al., 1992), peripheral vascular (Zelis and Flaim, 1982) and peripheral skeletal muscle (Sullivan et al., 1990) changes brought about by decreased cardiac function and physical inactivity. Following cardiac transplantation, patients with end-stage heart failure have a marked improvement in functional class (Pennock et al., 1982) and exercise capacity (Marzo et al., 1992). Although the initial and perhaps major contributor to increased exercise capacity is related directly to enhanced cardiac performance, normalization of the neurohormonal activation, (Levine et al., 1986; Olivari et al., 1987), pulmonary function (Ravenscraft et al., 1993) and peripheral vascular compliance (Arnold et al., 1989; Sinoway et al., 1988) after transplantation may also contribute. While skeletal muscle alterations in patients with chronic heart failure are believed to play an important role in the decreased exercise capacity (Sullivan et al., 1990; Minotti and Massie, 1992a), peripheral skeletal muscle recovery, has not to date been described after cardiac transplantation.

Decreased muscle strength (Lipkin et al., 1988) and enhanced muscle fatigue (Minotti et al., 1992b) have been observed in patients with chronic heart failure. In addition specific morphologic, biochemical and metabolic alterations have been described in these patients. Studies using ^{31}P NMR spectroscopy

have demonstrated abnormal skeletal muscle metabolism in these patients with early depletion of phosphocreatine and a rapid decrease in intracellular pH (Mancini et al., 1989; Massie et al., 1987; Wiener et al., 1986). More recently, skeletal muscle biopsy studies have revealed specific intrinsic skeletal muscle changes such as fibre atrophy, an increased percentage of type II fibres and decreased oxidative enzyme activity (Mancini et al., 1989; Sullivan et al., 1990). In view of the marked skeletal muscle adaptations to physical conditioning in healthy humans (Gollnick et al., 1973; Klausen et al., 1981), we hypothesized that changes in peripheral skeletal muscle after transplantation may contribute to increased exercise capacity. In the present study, we investigate the time course and magnitude of the skeletal muscle changes after cardiac transplantation using skeletal muscle biopsies of the vastus lateralis.

4.2 METHODS

4.2.1 Patient population

Eleven male patients (mean age 49±3 years, range 33-60) who underwent successful cardiac transplantation and survived the first post operative year were included in the study. All patients were in New York Heart Association functional class III to IV prior to transplantation. The etiology of end-stage heart failure was coronary artery disease in five patients, cardiomyopathy in five and valvular heart disease in one patient. All patients were treated with an optimal anti-failure regimen while awaiting cardiac transplantation including diuretics, angiotensin converting enzyme inhibitors and digoxin. After transplantation, the immunosuppressive regimen consisted of prednisone and cyclosporine to which azathioprine was added in three patients at one month, four patients at three months, and five patients at 12 months. The mean dose of prednisone was 22±1 mg, 19±1 mg and 8±1 mg at one, three and 12 months respectively. Other

medications in the post operative period included diuretics (three at one, three, and 12 months) and calcium channel blockers (one at one and three months, and five at 12 months). Supine resting right heart hemodynamic measurements were obtained prior to and at one to two weeks, three and 12 months after transplantation in all patients as previously described (Pflugfelder et al., 1988).

All patients participated in a physical rehabilitation program, in the first three months after transplantation. Rehabilitation consisted of rapid mobilization with bed exercises in the first post-operative week. Thereafter, and up to three months a more structured exercise program (five mornings/week) was initiated. The exercise regimen consisted of supervised stationary cycling, treadmill exercise, stair climbing, upper and lower extremity strengthening as well as stretching exercises. After three months, there was no organized rehabilitation program but, patients were encouraged to maintain fitness and received instructions and personal recommendations to continue exercise training at home.

4.2.2 Skeletal muscle biopsy

Subjects underwent a biopsy of the vastus lateralis muscle using the needle technique of Bergstrom (1962) prior to and at three and 12 months post transplantation. One patient did not have a biopsy taken prior to transplantation. In the remaining patients the biopsy was performed on average 39±3 days prior to transplantation. Six patients had an additional biopsy performed at 1 month post transplantation. At each time interval, two biopsy samples were obtained; one was put immediately in liquid nitrogen and was used for enzyme activity analysis. The second was mounted in embedding media (OCT), for histologic analyses, before being placed in liquid nitrogen. All biopsies were performed within 1 week of cardiopulmonary exercise testing. All samples were transferred to a freezer and stored at -70°C until analysis.

Histology and histochemistry. Samples were cut into 10μm sections at -20°C using a cryostat (Leitz). Serial sections were then stained for myofibrillar ATPase for fibre identification (Padykula and Herman, 1955). Fiber distribution was determined by simultaneously analyzing photographs of three serial sections of the same fibres stained for myofibrillar ATPase after pre-incubation at either pH 10.1, 4.6 and 4.3. Fibres were counted visually (average 110 fibres per sample) and the percentage of fibre type I, IIa and IIb determined. Very few type IIc fibres were found (<1% of the total), and therefore were not included in the analysis. Fibre size was determined on sections stained for nicotinamide adenine dinucleotide (NADH) tetrazolium reductase (Novikoff et al., 1961) by tracing the perimeter of at least 20 individual fibres per type using a calibrated digitizer (BQ Mug IV system). Muscle capillarization was determined using the amylase and periodic acid Schiff (PAS) procedure (Andersen, 1975). Photographic slides of these sections were projected, the number of capillaries in contact with each fibre type was counted and the number of capillaries in contact per fibre area calculated.

Skeletal muscle enzyme activity. Enzymatic assays were performed on tissue homogenates using a temperature controlled spectrophotometer (Philips) at 25°C. Activity of the glycolytic enzyme phosphofructokinase (PFK) was determined by means of the technique of Shonk and Boxer (1964). Activity of mitochondrial enzyme citrate synthase (CS) was determined using the method of Srére et al. (1969), while the activity of ß-hydroxyacyl CoA dehydrogenase (HAD), a mitochondrial enzyme involved in fatty acid oxidation, was determined as per the method of Bass et al. (1969). All of the homogenate assays for a given enzyme were conducted on the same day. All enzyme activities are expressed as μmol/g wet weight/min.

4.2.3 Cardiopulmonary exercise test

A symptom limited maximum upright exercise test was performed on an electrically braked cycle ergometer. Seven patients were well enough to undergo exercise testing before surgery. After transplantation the exercise test was repeated at three and 12 months. The initial workload was 12.5 or 25 Watts depending on the patient's ability to exercise. The workload was increased by 12.5 or 25 Watts every three minutes until fatigue. During the exercise test mixed expired gases were analyzed continuously for O_2 and CO_2 content. Ventilatory threshold was determined by the V-slope method of Beaver et al., 1986).

4.2.4. Statistical analyses

Data are presented as means ± SEM. Hemodynamics measurements and skeletal muscle changes before and after transplantation were examined using a repeated measures oneway analysis of variance. Skeletal muscle biopsies performed at 1 month were compared to pre-transplant biopsies using two-tailed paired t-test. Pre transplantation cardiopulmonary exercise test parameters were compared to the 3 and 12 months values using a one way analysis of variance.

4.3 RESULTS

Skeletal muscle biopsy histology. Patients had a predominance of type II fibres (65±3 percent) before transplantation. Fibre type distribution did not change after transplantation (Figure 7). Type I, type IIa and type IIb fibre cross sectional areas increased significantly from pre-transplant values by 38 %, 39 %, and 47 % respectively by 12 months post transplantation (Figure 8, Table 5).

Prior to transplantation, the number of capillaries adjacent to type I fibres was greater than the number adjacent to type IIa and IIb fibres (Table 6). After transplantation, the number of capillaries adjacent to individual fibres did not change significantly (Table 6). However, since the fibre size increased

significantly after transplantation, there was a concomitant decrease in the number of capillaries per fibre area which was significant in type 1 fibres by 12 months post transplantation (Table 6).

Skeletal muscle enzymes. The activity of phosphofructokinase increased significantly by three months post transplantation and remained the same at 12 months. The increase in citrate synthase and ß hydroxyacyl CoA dehydrogenase activity was significant at 12 months only (Table 5). Phosphofructokinase, citrate synthase and ß hydroxyacyl CoA dehydrogenase increased on average by 25 %, 47 % and 63 % respectively by 12 months.

In the six patients, with an additional biopsy at 1 month post transplantation, there was no significant changes in enzyme activities and fibre cross sectional area when compared to pre transplantation values (Table 7).

Resting hemodynamics and exercise test. There was a marked decrease in resting supine right atrial and pulmonary capillary wedge pressures and increase in cardiac index in the first week after transplantation with no further changes thereafter (Table 8). Peak oxygen uptake expressed in L/min increased significantly by three months post transplantation with a further increased by 12 months. Because of the increase in body weight by 12 months post transplantation the peak oxygen uptake expressed in ml/kg/min increase in the first three months post transplantation only (Table 8).

Table 5. Skeletal muscle fibre cross sectional areas and enzyme activities before and at three, and 12 months following cardiac transplantation

	Pre n=10	3 months n=10	12 months n=11
Type I CSA (μm^2)	3070±153	3459±238	4223±238*
Type IIa CSA (μm^2)	2661±149	3306±270	3703±265*
Type IIb CSA (μm^2)	2039±175†	2532±215†	2989±264*†
PFK (µmol/g ww/min)	28.7±1.6	34.4±2.1*	35.9±1.8*
CS (µmol/g ww/min)	4.3±0.2	5.3±0.6	6.3±0.4*
HAD (µmol/g ww/min)	3.5±0.3	5.0±0.6	5.7±0.6*

Means±SEM; CS=citrate synthase; CSA=cross sectional area; HAD=ß-hydroxyacyl CoA dehydrogenase; PFK=phosphofructokinase

*p<0.05 vs pre; † p<0.05 vs type I and IIa

Table 6. Number of capillaries surrounding each skeletal muscle fibre types and the number of capillaries per fibre area before and at three, and 12 months following cardiac transplantation

	Pre n=10	3 months n=10	12 months n=11
Number of Cp per fibre			
Type I	4.51±0.09	4.42±0.11	4.57±0.14
Type IIa	3.96±0.10†	4.07±0.08†	4.09±0.15†
Type IIb	3.11±0.14††	3.13±0.10††	3.39±0.15††
Cp/ fibre area ($Cp/\mu m^2 x10^{-3}$)			
Type I	1.51±0.07	1.37±0.13	1.13±.0.09*
Type IIa	1.49±0.07	1.35±0.15	1.19±0.13
Type IIb	1.62±0.15	1.32±0.10	1.26±0.15

Means±SEM; Cp=capillaries

* $p<0.05$ vs pre; † $p<0.05$ vs Type I; †† $p<0.05$ vs Type I and Type IIa

Table 7. Skeletal muscle fibre cross sectional areas and enzyme activities in six patients before and at one month following cardiac transplantation

	Pre n=6	1 month n=6
Type I CSA (μm^2)	2781±258	2832±615
Type IIa CSA (μm^2)	2715±245	2575±408
Type IIb CSA (μm^2)	2266±294	2131±395
PFK (µmol/g ww/min)	30.1±2.3	33.1±4.5
CS (µmol/g ww/min)	4.6±0.5	4.7±0.7
HAD (µmol/g ww/min)	3.7±0.6	4.3±0.8

Means±SEM; CS=citrate synthase; CSA=cross sectional area; HAD=ß-hydroxyacyl CoA dehydrogenase; PFK=phosphofructokinase

Table 8. Resting hemodynamics and peak exercise test results before and at three, and 12 months following cardiac transplantation

	Pre n=11	3 months n=11	12 months n=11
Resting RAP (mmHg)	16±2	7±1*	5±1*
Resting PCWP (mmHg)	27±1	13±1*	15±2*
Resting CI (L/min/m^2)	1.6±0.2	3.2±0.2*	3.0±0.2*
Weight (kg)	75±3	73±3	82±3**
VO_2 max (L/min) †	0.74±0.05	1.31±0.11*	1.61±0.20**
VO_2 max (ml/kg/min) †	9.8±0.5	17.4±1.5*	19.5±2.4*
VT (ml/kg/min)†	7.4±0.6	10.5±0.7*	12.0±1.3*
PeakHR (beats/min) †	100±7	124±6*	131±5*
Peak BP sys (mmHg) †	94±7	178±8*	181±5*

Means±SEM; † n=7 for exercise test results

BP sys= systolic blood pressure; CI=cardiac index; HR=heart rate; PCWP=pulmonary capillary wedge pressure; RAP=right atrial pressure; VO_2 max=peak oxygen uptake; VT=ventilatory threshold

* $p<0.05$ vs Pre; ** $p<0.05$ vs Pre and 3 mo

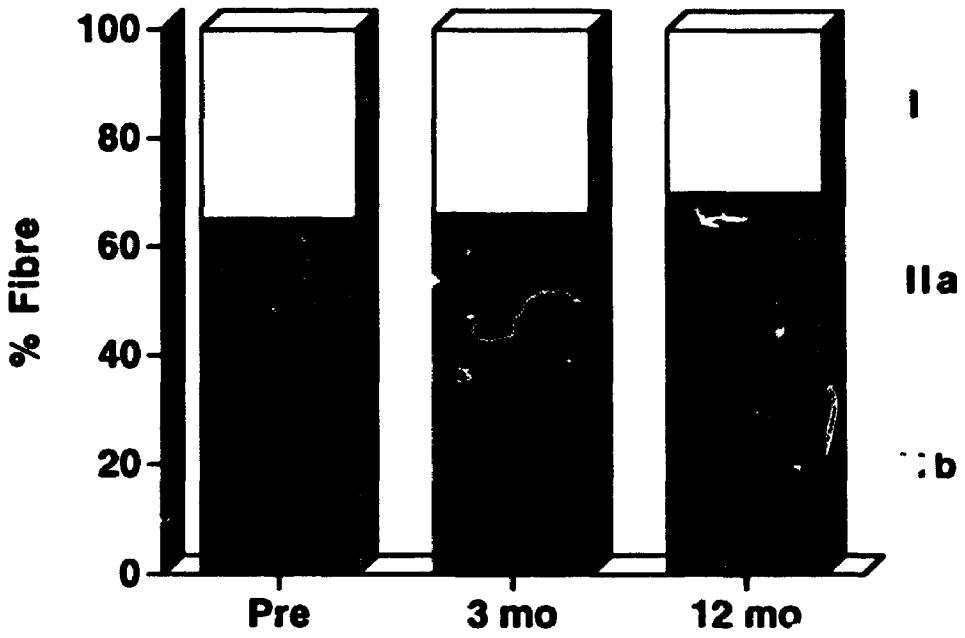


Figure 7. Skeletal muscle fibre type distribution before and at three, and 12 months following cardiac transplantation.

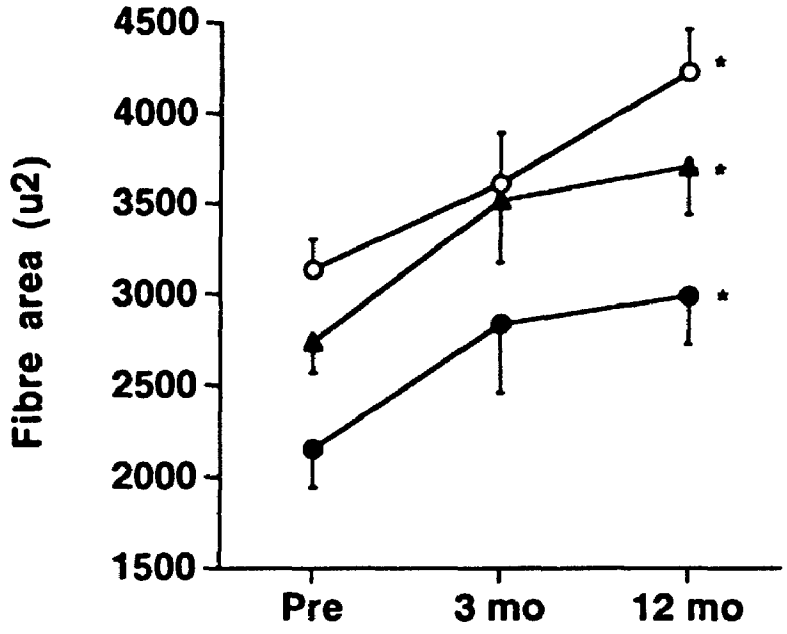


Figure 8. Skeletal muscle type I (open circle), type IIa (closed triangle) and type IIb (closed circle) fibre cross sectional areas before and at three, and 12 months following cardiac transplantation. Values are means±SEM. * Significantly different from pre transplantation ($p<0.05$)

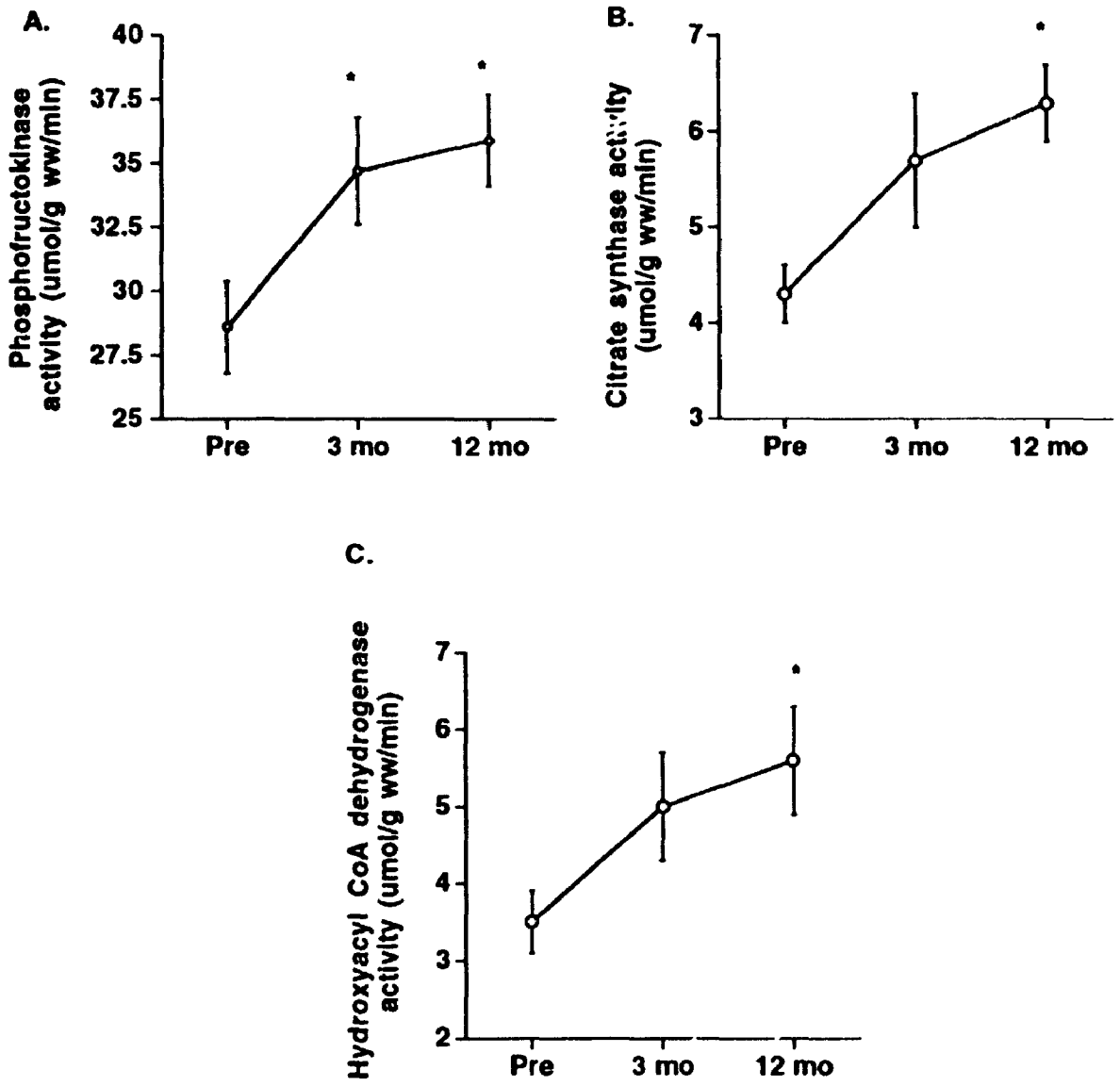


Figure 19. Skeletal muscle phosphofructokinase (A), citrate synthase (B), and ß-hydroxyacyl CoA dehydrogenase (C) activities before and at three, and 12 months following cardiac transplantation. Values are means±SEM. * Significantly different from pre transplantation ($p<0.05$).

4.4 DISCUSSION

Skeletal muscle histological (Lipkin et al., 1988; Mancini et al. 1990; Sullivan et al. 1990), biochemical (Mancini et al. 1990; Sullivan et al. 1990) and metabolic (Massie et al., 1987; Wiener et al., 1986) alterations have been observed in patients with chronic heart failure and are believed to play an important role in the early muscle fatigue and decreased exercise capacity of patients with heart failure (Drexler 1992; Mancini et al. 1990; Poole-Wilson et al. 1988; Sullivan et al. 1991; Wiener et al., 1986). Previous studies using ^{31}P NMR spectroscopy have shown substantial normalization of skeletal muscle metabolism in patients with chronic heart failure after exercise training (Adamopoulos et al., 1993; Minotti et al., 1990). The present results suggest that the histological and biochemical abnormalities are also in part reversible in patients with end-stage heart failure after cardiac transplantation. Skeletal muscle fibre cross sectional area and oxidative enzyme activity increased after cardiac transplantation and these adaptations were associated with an increased exercise capacity. These results provide further evidence for the possible role of skeletal muscle alterations in the pathophysiology of exercise intolerance in chronic heart failure.

Vasodilator therapy in patients with chronic heart failure improved central hemodynamics and peripheral blood flow acutely, but does not result in an immediate increase in exercise capacity, however exercise capacity improves over time (Drexler et al., 1988; Kramer et al., 1983). These findings support the important role of the periphery in limiting exercise capacity and suggest a slow recovery for these peripheral changes. Normalization of resting central hemodynamics occurs within one to two weeks after cardiac transplantation (Bhatia et al., 1987) whereas peak oxygen uptake improvement occurs over time in the early post operative period (Bussières et al., 1991).

Cardiac cachexia has been described in patients with severe chronic heart failure. This form of malnutrition which is due to inactivity, anorexia and hypermetabolism results in profound reduction of muscle and adipose tissue mass (Mancini et al., 1992; Pittman and Cohen, 1964). In patients with long-term heart failure, gastrocnemius biopsies have revealed atrophy of predominantly type IIa and type IIb fibres (Mancini et al., 1989). The findings in the vastus lateralis muscle, however, have been inconsistent with either atrophy of type II fibres (Lipkin et al., 1988; Sullivan et al., 1990; Yancy et al., 1989), or an hypertrophy of type I (Minotti et al., 1993) when compared to normals. We have observed reduced fibre cross sectional areas in all fibre types. The fibre cross sectional areas were on average equal to less than 75 % of normals values (Gollnick et al., 1973; Klausen et al., 1981) with the most severe atrophy observed in the type IIb fibres. The universal and pronounced fibre atrophy observed in our patients might be explained by their more severe condition, as all patients had advanced heart failure and were awaiting cardiac transplantation. Pronounced fibre atrophy has also been reported by Wilson et al. (1992) in an experimental canine model of heart failure. The potential mechanisms for skeletal muscle atrophy in chronic heart failure are multiple and include deconditioning (Minotti and Massie, 1992a), decreased caloric and protein intake (Mancini et al., 1992; Carr et al., 1989), decreased skeletal muscle blood flow (Zelis and Flaim, 1982), increased free radical formation (Belch et al., 1991), and increased catecholamines (Cohn et al., 1984; Francis, 1990), and tumor necrosis factor (Levine et al., 1990).

After cardiac transplantation, the fibre cross sectional area increased by 38-47 % but this increase was not significant until 12 months post-transplantation. The delay in the increase in fibre areas might have been due to the high doses of corticosteroids given in the first months after transplantation. High dose

corticostercid therapy has been associated with fibre atrophy in normal muscle (Ruff, 1986). Increased caloric intake and more efficient energy utilization due to the relief of hepatic and portal vein congestion after transplantation, in addition to a diminution in basal metabolic rate, the reversal of catabolic factors and increase physical activity could be responsible for the gain in skeletal muscle fibre cross sectional area after surgery. Fibre cross sectional area has been shown to increase in normals (Andersen and Henriksson, 1977; Gollnick et al., 1973) and in patients with angina pectoris (Ferguson et al., 1982) after exercise training .

Prior to transplantation, our patients had a predominance of type II fibres when compared to normals. The increased percentage of type II fibres has been observed by others in patients with chronic heart failure (Mancini et al., 1989; Sullivan et al., 1990) and angina pectoris (Karlsson, 1987). The cause of the alteration in fibre distribution in patients with cardiac disease is unknown but could be the result of selective type I cell death due to increased free radical formation in these oxidative fibres (Belch et al., 1991, Karlsson, 1988), transformation of type I fibres to type II fibres or/and a genetic predisposition in this population (Karlsson, 1988). After transplantation, we did not observe a change in fibre type distribution. Although it has been shown that electrical stimulation and cross reinervation cause changes in fibre distribution (Roy et al., 1991), the effect of some physiological stimuli, such as conditioning or deconditioning on fibre distribution in normal muscle in man, remains controversial. Short term endurance training changes the biochemical profile of type II fibres but not myofibrillar ATPase activity (Saltin and Gollnick, 1983). However, evidence from cross sectional population studies suggests that alterations in fibre composition might occur as a result of years of endurance training (Gollnick, 1972). It is possible that neither the duration nor the intensity of the rehabilitation in our program was effective to produce any fibre changes.

Similarly a six month exercise training program, in patients with angina pectoris, did not alter the fibre distribution (Ferguson et al., 1982).

Capillarization in skeletal muscles of patients with heart failure, has been reported to be increased (Mancini et al., 1989), unchanged (Lipkin et al., 1988) or decreased (Sullivan et al., 1990; Yancy et al., 1989; Drexler et al., 1992) when compared to normals. In these patients, capillaries are submitted to two stimuli bringing about opposite changes: deconditioning and ischemia. In normal males, the number of capillaries around each fibre decreases with deconditioning (Klaussen et al., 1981) whereas exercise conditioning has the opposite effect (Andersen and Henriksson, 1977; Gollnick et al., 1973; Klaussen et al., 1981). Ischemia on the other hand causes an increase in the number of capillaries around each fibre (Hammarsten et al., 1980). After transplantation, the skeletal muscle capillarization did not improve since while the number of capillaries around each fibre did not change significantly after transplantation the number of capillaries per fibre area decreased due to the significant increases in fibre size. The number of capillaries around each fibre however remained above the reported normal value of 0.8-1.1 capillaries per μm^2 $x10^{-3}$ (Andersen and Henriksson, 1977; Sullivan et al., 1990).

In patients with end-stage heart failure, decreased oxidative enzyme concentrations and activities resembling the changes observed with deconditioning have been reported (Mancini et al., 1989; Sullivan et al., 1990). In the present study a significant increase in glycolytic and oxidative enzyme activities was found after cardiac transplantation. While endurance training of moderate to high intensity has been shown to augment the activity of oxidative enzymes (Andersen and Henriksson, 1977; Gollnick et al., 1973; Klaussen et al., 1981), only high intensity training results in increased phosphofructokinase activity (Gollnick et al., 1973). The significant increases in enzyme activities after

transplantation suggest that increased physical activity after transplantation in our patients was a strong enough stimulus to increase skeletal muscle metabolic capacity in these patients.

Despite marked improvement in skeletal muscle morphology after transplantation, the skeletal muscle fibre cross sectional areas in the present study were still lower than the reported normal values of 4500 to 6500 μm^2 at 12 months. The incomplete normalization of fibre cross sectional areas coupled with the modest increase in oxidative enzymes activities after transplantation, when compared to a training study (Gollnick et al., 1973) suggest that more aggressive physical rehabilitation post surgery could benefit these patients. Persistence of skeletal muscle abnormalities post transplantation may play an important role in the decreased exercise capacity observed in these patients when compared to age-matched controls (Kavanagh et al., 1988; Savin et al., 1980) and predicted values (Bussières-Chafe et al., 1993).

CHAPTER FIVE

BASIS FOR THE AEROBIC IMPAIRMENT IN PATIENTS LATE POST CARDIAC TRANSPLANTATION

5.1 INTRODUCTION

The survival after cardiac transplantation has increased to values above 80% at one year (Kaye, 1992) due to improvements in the last decade in the detection and treatment of rejection, in addition to better patient selection and post-operative care. Because of the increasing number of patients surviving the operation, the long term benefit of this procedure is now being evaluated. Functional class (Pennock et al., 1982) and exercise capacity improve markedly in patients with end-stage heart failure following cardiac transplantation yet, the peak oxygen uptake remains low at about 2/3 of aged matched controls (Brubaker et al., 1993; Kavanagh et al., 1988; Savin et al., 1980). The basis for this limited exercise tolerance has not been well characterized though, both central and peripheral abnormalities are believed to be important. Possible mechanisms include, severe deconditioning from extended periods of bed rest before transplantation (Savin et al., 1980), cardiac dysfunction from damage during the peri- or post operative periods (Labovitz et al., 1989), cardiac denervation, peripheral vasoconstriction due to cyclosporine therapy ((Golub and Berger, 1987)) and skeletal muscle myopathy due to prednisone therapy. The present investigation was undertaken to determine the physiological basis for the suboptimal exercise performance observed in late cardiac transplant recipients.

5.2 METHODS

5.2.1 Patient population

Fifty-seven cardiac transplant recipients were studied at the time of routine follow up, one to nine years (mean 2.3±0.2 years) following cardiac transplantation. The recipients were predominantly male (male=54, female=three), with a mean age at the time of evaluation of 50±2 years (range 19-62). The etiology of end-stage heart failure was coronary artery disease in 33, cardiomyopathy in 20, valvular heart disease in three and congenital heart disease in one. All patients were receiving prednisone and cyclosporine at a mean dose of 5.2±0.3 mg and 350±15 mg per day respectively. To this regimen azathioprine was added in 32 patients at a mean dose of 100 mg per day. In addition, 41 patients used either one or two of the following antihypertensive agents: calcium channel blockers (n=25), angiotensin converting enzyme inhibitors (n=11), and diuretics (n=12).

5.2.2. Cardiopulmonary exercise testing

All patients underwent an upright graded cycle exercise test with mixed expired gas analysis and ventilatory measurements. The initial workload was 25 Watts and the workload was increased by 25 Watts every 3 minutes until the point of fatigue. Mixed expired gases were analyzed continuously for O_2 and CO_2 content (Ametek analyzers). Data were processed on line by a microcomputer (IBM) programmed to correct for delays between signals for volume and gas concentration and to present the average values during every 10-15 second interval for the following variables: minute ventilation, respiratory rate, oxygen uptake, carbon dioxide output, respiratory exchange ratio and heart rate. The ventilatory (anaerobic) threshold was determined by the V-slope method (Beaver et al., 1986). Arterial blood pressure was measured using a sphygmomanometer at rest and toward the end of each exercise stage. In 36

patients concomitant central hemodynamic measurements were obtained during exercise. After routine endomyocardial biopsy, a flow directed thermodilution catheter was introduced through the internal jugular vein and advanced into a branch of the right pulmonary artery for hemodynamic measurements at rest and during each exercise stage as previously described (Pflugfelder et al., 1988). Thermodilution cardiac outputs were measured in duplicate or triplicate at the end of each exercise stage to obtain values which differed by less than 10%. In the remaining patients resting supine central hemodynamics were obtained within 7 days of the exercise test.

In order to correct for the decreased peak oxygen uptake observed with aging, the functional aerobic impairment was used to standardize the peak oxygen uptake. The formula employed was the following; FAI= [(VO_2 max predicted - VO_2 max observed) / VO_2 max predicted] x 100 (Bruce et al., 1974). Therefore, a value >0 is indicative of a performance less than predicted whereas a value <0 is indicative of a better performance than predicted. Predicted values were obtained from Jones' equations (1988).

5.2.3 Statistical analysis

Data are expressed as means ± SEM. To evaluate the basis for the decreased exercise capacity in late cardiac transplant recipients, the relationship between functional aerobic impairment (FAI) and pre-operative, intra-operative, and post-operative factors and maximal exercise test parameters was determined using a linear regression model. A p value <0.05 was considered statistically significant

5.3 RESULTS

Exercise test. The maximal exercise test results are shown in Table 9. The mean peak oxygen uptake was 21.7±0.9 ml/kg/min. The ventilatory threshold, determined by the V-slope method, occurred, on average, at 55% of peak oxygen uptake. Peak respiratory exchange ratio was 1.14±0.01 and above 1.00 in all patients. Peak heart rate was 142±2 beats/min. There was a relationship between peak oxygen uptake and age (Figure 10).

The average functional aerobic impairment (FAI) was 34±2% and the peak oxygen uptake was, on average, 66% of the predicted values. The distribution of the functional aerobic impairment is shown on Figure 10. Forty patients had values between 20 and 50% However, six patients had exercise capacity comparable to normal (FAI values < 10) whereas two patients were markedly impaired (FAI > 60%).

Relation between FAI and pre-, intra-, and post-operative factors. The relation between the functional aerobic impairment and pre-operative factors are shown on Table 10. The duration of disease correlated modestly with the FAI (Figure 11A). There was no significant difference in the FAI for the different etiologies of heart failure. The average FAI in patients with an initial diagnosis of coronary artery disease was 34±3% compared to 33±4% in patients with cardiomyopathy (Figure 11B). Similarly, there was no significant correlation between FAI and central hemodynamics prior to transplantation (Table 10).

There was no correlation between FAI and intra-operative factors such as age at time of transplantation, total ischemic time, donor age, height, gender or donor:recipients weight ratio (Table 10). Similarly there was no correlation between FAI and any index of resting cardiac function measured within days of the exercise test. Likewise, body mass index, an indicator of body composition, did not correlate with FAI (Table 10). Other recipient characteristics at the time of

exercise testing analyzed such as age at time of exercise, time post transplantation, number of rejection episodes, dose of immunosuppressive agents, or antihypertension therapy did not correlate with FAI. Fifteen patients had evidence of coronary artery disease, as defined by a stenosis of greater than 30%, on an angiogram performed within days of the exercise test. There was no correlation between the FAI and the presence of coronary artery disease.

Relation between FAI and peak exercise test parameters. There was a strong correlation between the peak cardiac index and arterio-venous oxygen difference and the FAI (Figure 12A and B). Likewise, peak heart rate and peak systemic vascular resistance were also related to FAI (Figure 12C and D). Of note, the peak systemic vascular resistance was strongly related to the peak CI (Figure 13A) while peak pulmonary capillary wedge pressure did not correlate with peak cardiac index (Figure 13B). There was also a correlation between the peak heart rate and stroke volume index and FAI (Table 11). However, no correlation was found between peak mean blood pressure and peak right atrial and pulmonary capillary wedge pressures and FAI.

Table 9. Peak exercise test results of 57 patients late post cardiac transplantation

Peak oxygen uptake (ml/kg/min)	21.7±0.9
Ventilatcry threshold (ml/kg/min)	12.3±0.4
Peak respiratory exchange ratio	1.14±0.01
Peak heart rate (beats/min)	142±2
Functional aerobic impairment (%)	34±2
Peak pulmonary capillary wedge pressure (mmHg) n=36	15±1
Peak cardiac index (L/min/m^2) n=36	6.2±0.2
Peak a-v O_2 difference(ml/L) n=36	130±3

Means±SEM

Table 10. Regression coefficients for the relationship between functional aerobic impairment and some pre- and intra-operative factors in 57 patients late post cardiac transplantation

	r	p value
Pre-operative resting hemodynamics		
Right atrial pressure (mmHg)	0.05	0.72
Pulmonary capillary wedge pressure (mmHg)	0.04	0.75
Cardiac index (L/min/m^2)	-0.10	0.49
Intra-operative factors		
Recipient's age at transplantation	0.04	0.79
Total ischemic time (min)	-0.20	0.14
Donor age (yrs)	0.01	0.94
Donor:recipient weight ratio	-0.11	0.41

Table 11. Regression coefficients for the relationship between functional aerobic impairment and some post-operative factors and peak exercise test parameters in 57 patients late post cardiac transplantation

	r	p value
Age at time of exercise (yrs)	0.01	0.94
Time post transplantation (yrs)	-0.17	0.21
Body mass index (kg/m^2)	0.09	0.53
Rejection episodes (#)	0.20	0.14
Peak exercise test results (n=36)		
Heart rate (beats/min)	-0.39	0.003*
Mean blood pressure (mmHg)	-0.17	0.24
Right atrial pressure (mmHg)	0.08	0.60
Pulmonary capillary wedge pressure (mmHg)	0.28	0.10
Stroke volume index (ml/m^2)	-0.44	0.007*

* $p<0.05$

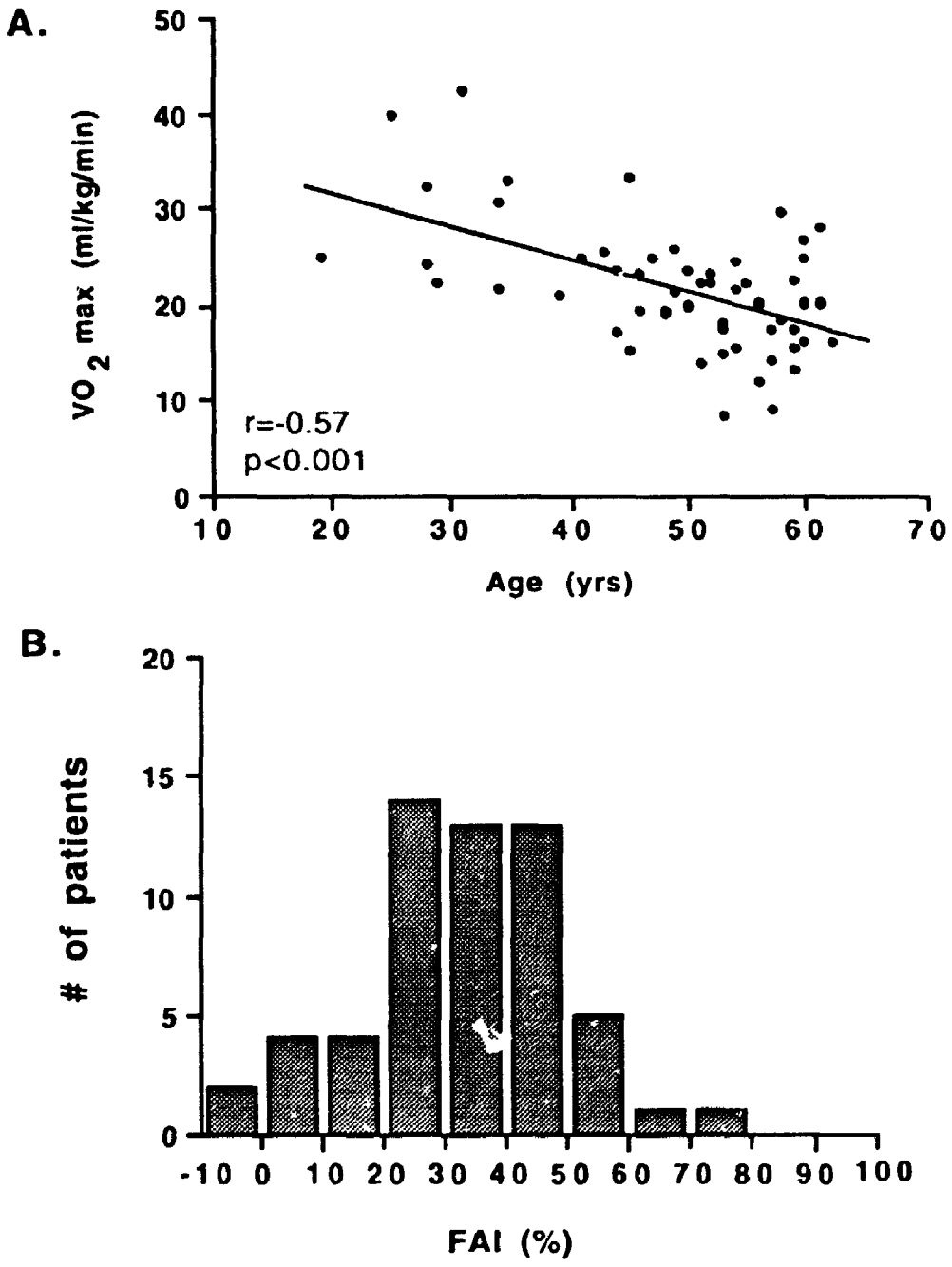


Figure 10. Relationship between age at time of the exercise test and peak oxygen uptake (A). The distribution of the functional aerobic impairment (B) in 57 patients late post cardiac transplantation.

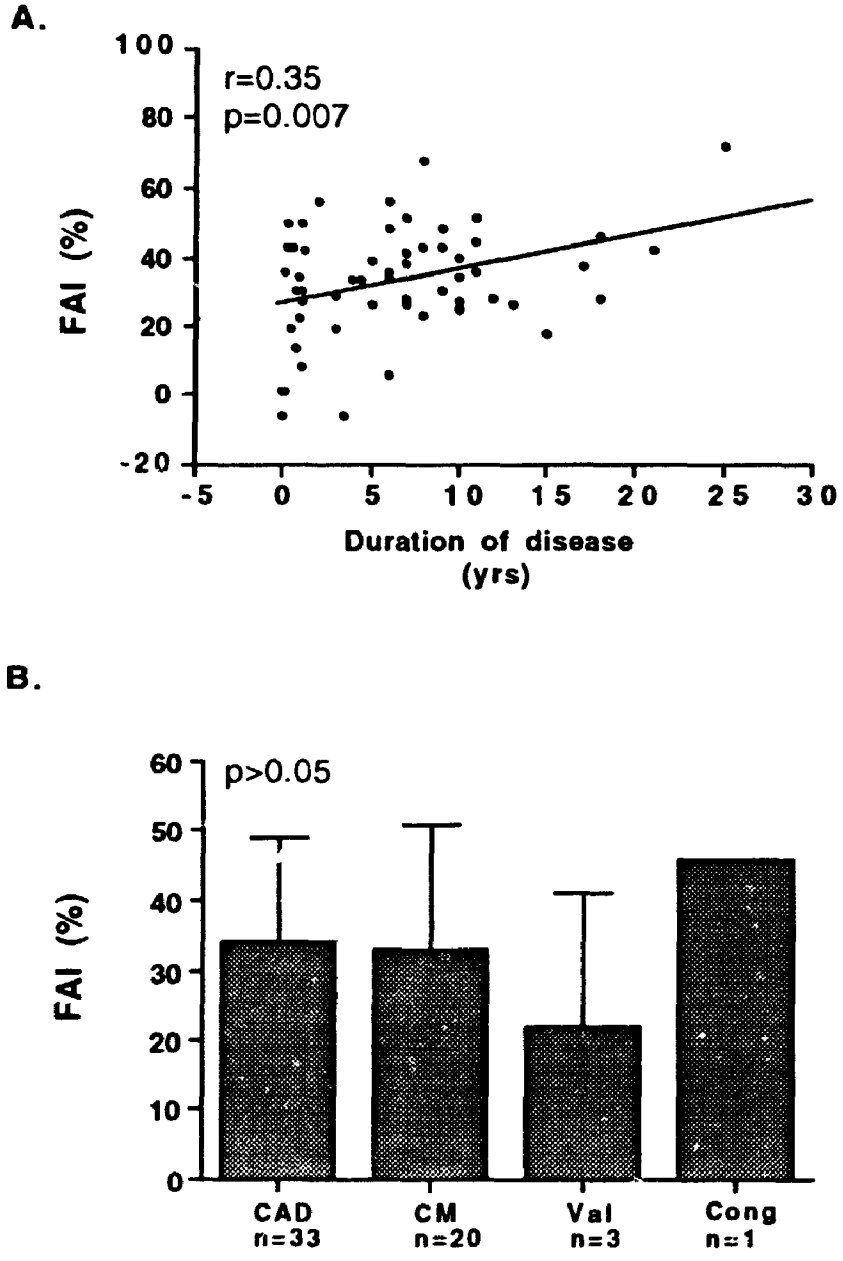


Figure 11. Relationship between duration of cardiac disease and functional aerobic impairment (A). The average functional aerobic impairment for the different etiologies of heart failure (B). Values are means±SE. (CAD=coronary artery disease; CM=cardiomyopathy; Val=valvular heart disease; cong=congenital heart disease).

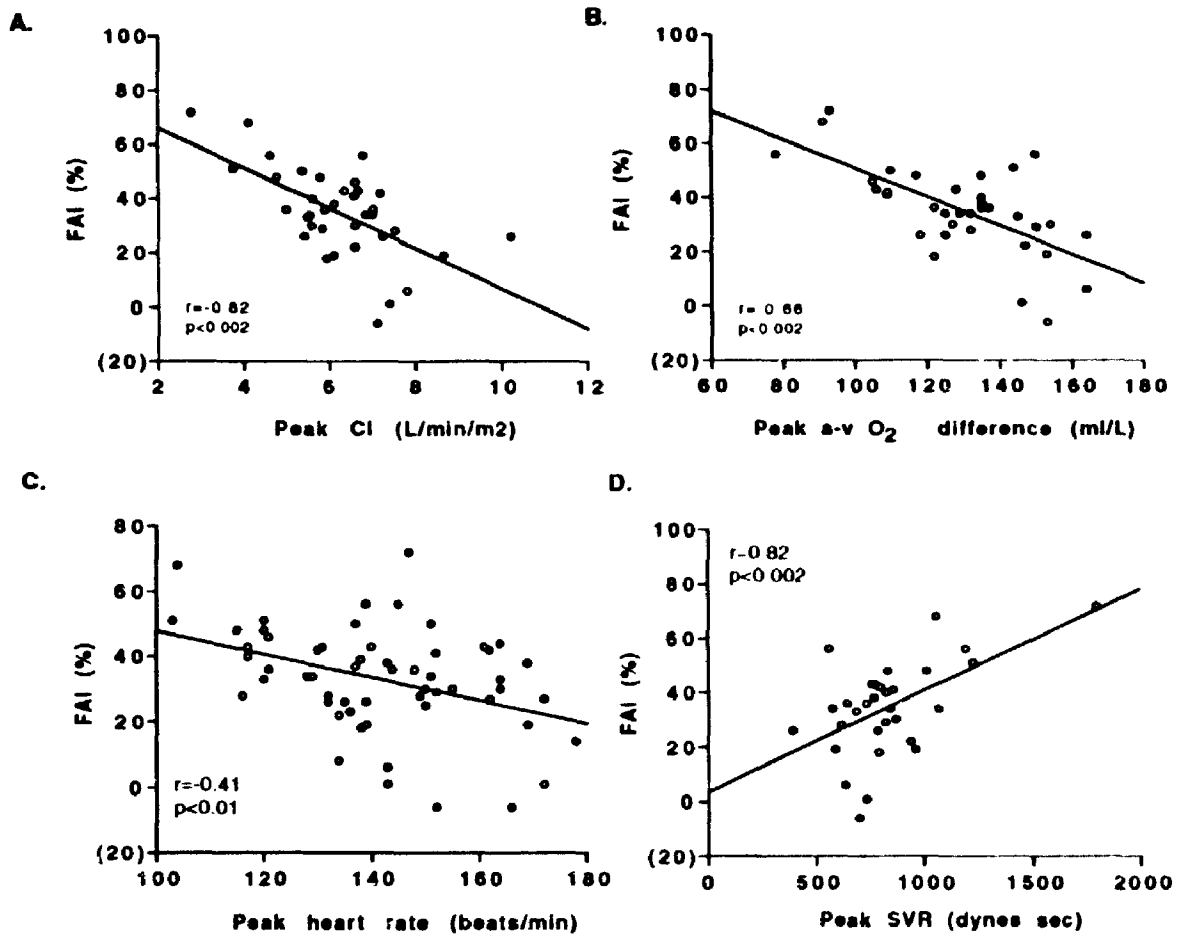


Figure 12. Relationship between functional aerobic impairment and peak cardiac index (A), peak arterio-venous oxygen difference (B) and peak heart rate (C) and peak systemic vascular resistance (D) in 36 patients late post cardiac transplantation.

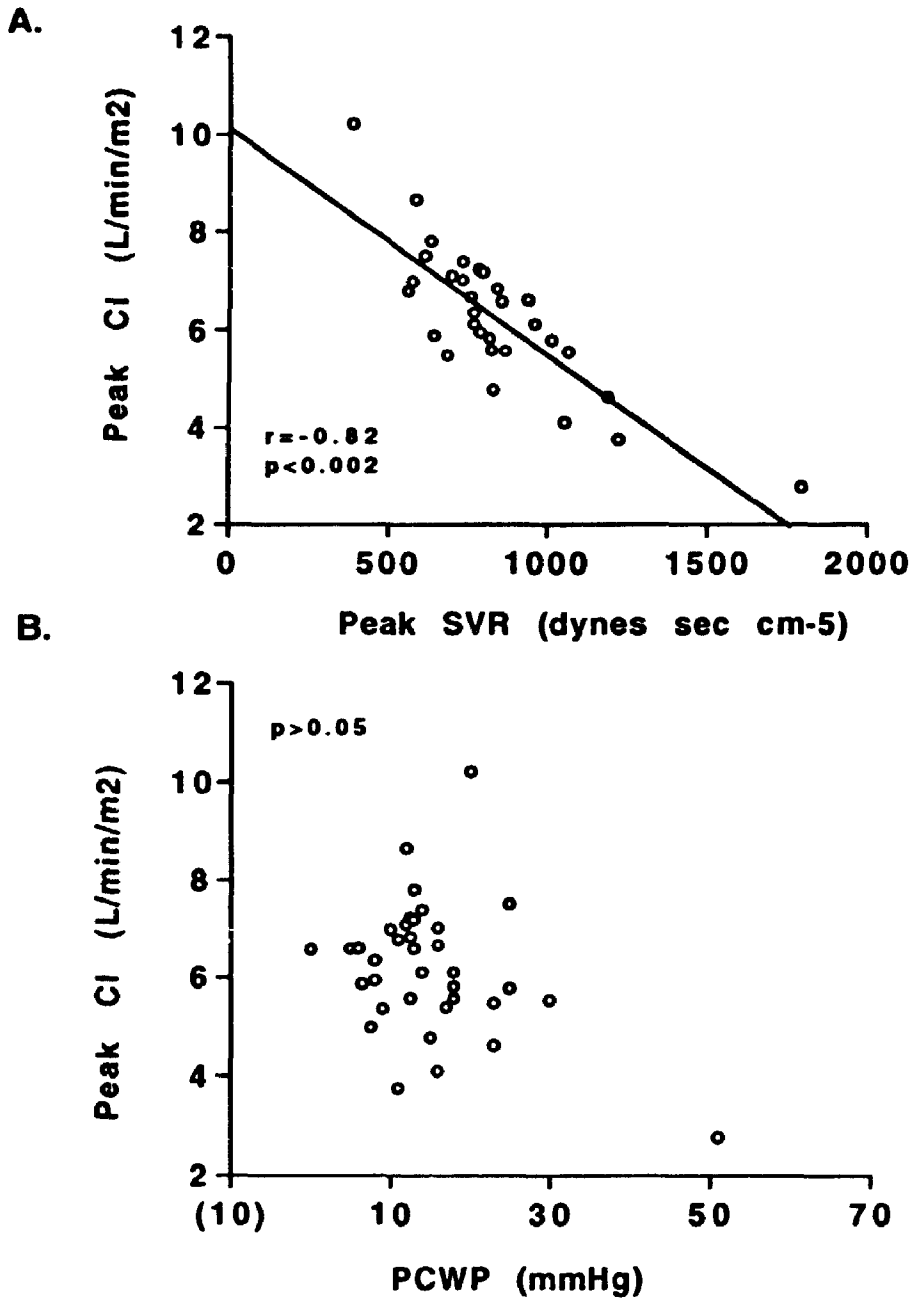


Figure 13. Relationship between peak cardiac index and peak systemic vascular resistance (A) and peak pulmonary capillary wedge pressure (B) in 36 patients late post cardiac transplantation.

5.4 DISCUSSION

Earlier work on exercise capacity after cardiac denervation has reported little or no deterioration in exercise capacity in regionally cardiac-denervated dogs (Donald et al., 1964a). In contrast, studies in humans after cardiac transplantation have found earlier onset of fatigue and decreased work capacity than age-matched controls or predicted values (Brubaker et al., 1993; Kavanagh et al., 1988; Labovitz et al., 1989; Savin et al., 1980). In analyzing the difference between the response of the denervated animals and human cardiac transplant recipients one must consider, in addition to cardiac denervation, the state of the patients prior to transplantation (Savin et al., 1980), the effects of graft retrieval (Labovitz et al., 1989), and rejection (Labovitz et al., 1989; Savin et al., 1980) on cardiac function, and the repercussions of immunosuppressive drugs (Golub and Berger, 1987) or others medications post transplantation.

In the present study, the peak oxygen uptake of late cardiac transplant recipients was on average 21.7 ml/kg/min and it corresponded to 2/3 of that predicted for age and sex (Jones 1988). The value for the peak oxygen uptake was similar to that observed by others in cardiac transplant recipients (Brubaker et al., 1993; Kavanagh et al., 1988; Labovitz et al., 1989; Savin et al., 1980). Whereas decreased peak oxygen uptake can be due to decreased patient effort, the high respiratory exchange ratio at peak exercise and the low ventilatory threshold as a percentage of peak oxygen uptake in the present study suggest that the exercise test was maximal and the decreased peak oxygen uptake was not the result of a submaximal exercise test or decreased patients motivation. Similarly, it is unlikely that the decreased exercise capacity was due to inadequate recovery from the surgical procedure itself, since the exercise test was performed at one or more years post transplantation in an attempt to

eliminate the effects of bed rest and complications in the early post-operative period.

In order to correct for the decreased exercise capacity reported in normals with aging, the peak oxygen uptake was standardized, in the present study, using functional aerobic impairment (FAI). The FAI represents the percentage difference between functional aerobic capacity observed with maximal exercise testing and that expected of healthy persons of similar sex and age (Bruce et al., 1974). In late cardiac transplant recipients the FAI is, on average, equal to 34%. Its etiology is potentially complex in origin in view of the possible pre-, intra-, and post-operative factors that can affect the exercise capacity in these patients.

Pre-operative exercise capacity is limited in patients with chronic heart failure due to cardiac dysfunction, neurohumoral activation (Francis, 1987), and peripheral abnormalities (Lipkin et al., 1988; Sullivan et al., 1990; Zelis et al., 1968). The persistence of these abnormalities, especially in patients with a long history of cardiac disease, could contribute to the decreased exercise capacity observed post transplantation. In agreement, we have found a correlation between the duration of cardiac disease and FAI. The FAI, however, was not affected by the etiology of end-stage heart failure. Although patients with end-stage heart failure, due to coronary artery disease, tend to be older than patients with cardiomyopathy, and have more of a systemic disease that could decrease the exercise capacity, we have not observed a poorer exercise performance in these patients late post transplantation. It is possible that the intrinsic skeletal muscle abnormalities observed in patients with cardiomyopathy contribute to their decreased exercise capacity late post transplantation (Dunnigan et al., 1987).

Intra-operative factors that could potentially decrease cardiac performance and thereby exercise capacity post transplantation included

cardiac preservation, donor characteristics and donor recipient size mismatch. An examination of the relation between FAI and some donor characteristics such as age, sex and donor:recipient weight ratio revealed none. Similarly, FAI did not correlate with total ischemic time. This finding is in contrast to the results of Labovitz et al. (1989) who have reported a correlation between total ischemic time and exercise capacity. This discrepancy could be explained by the incomplete recovery from intra-operative cardiac injury in their patients who were tested within the first year post transplantation. Our laboratory has observed improvement in cardiac function in the first year post transplantation with stabilization thereafter (Rudas et al., 1990). In keeping with the adequate cardiac recovery from global ischemia late post transplantation, we have observed normal resting hemodynamics in most patients late post transplantation with no correlation between indices of resting cardiac function, total ischemic time and FAI.

Carrier et al. (1986) have observed that, when carefully selected, older patients fare as well as younger patients in terms of survival after transplantation. The exercise capacity seems to exhibit similar trends since the age at the time of transplantation or the age at time of the exercise test did not correlate with FAI. Similarly, obesity did not seem to influence the exercise capacity. Many patients tend to be obese following transplantation, possibly due to increased appetite from long-term corticosteroid therapy (Grady et al., 1991; Hagan et al., 1990), obesity prior to their illness (Rubin et al., 1991), and/or lack of compliance with prescribed diet and exercise programs (Grady et al., 1991). Obese patients have a lower peak oxygen uptake than normals when corrected for body weight (Buskirk and Taylor, 1957). However, this relationship was not observed in cardiac transplant recipients since patients with a high body mass index did no worse than leaner patients. It is possible that the increased caloric intake

decreased the catabolic effect of prednisone on the skeletal muscle (Ruff, 1986) after transplantation. Greater increases in body weight could also reflect a better sense of well being post transplantation (Grady et al., 1991) which is associated with an increased exercise tolerance.

As in the denervated dogs, cardiac transplant recipients increased cardiac output during the early stages of exercise due to the Frank-Starling mechanism and in the later stages because of the effects of increased levels of circulating catecholamines (Pope et al., 1980). However, unlike the denervated dog (Donald et al., 1964b), at peak exercise, heart rate and cardiac output are considerably lower than normal (Ehrman et al., 1992; Quigg et al., 1989). Decreased cardiac output and exercise tolerance, in cardiac transplant recipients, are thought to be due to cardiac dysfunction possibly from cardiac ischemia, rejection, denervation and/or premature atherosclerosis. The present investigation however, suggests that peak oxygen uptake and peak cardiac output are not limited by cardiac dysfunction. Supporting this hypothesis is the present lack of correlation between functional aerobic impairment and peak right and left ventricular filling pressures. In addition, whereas patients with cardiac dysfunction have subnormal increases in cardiac output during exercise (Koike et al., 1992), a normal increase in cardiac output for a given oxygen uptake have been reported in cardiac transplant recipients during upright exercise (Kavanagh et al., 1988; Jensen et al., 1991). Thus, the correlation between peak cardiac index and peak systemic vascular resistance, observed in the present study, suggest that the maximal cardiac output and thereby peak oxygen uptake are, in part, limited by peripheral factors.

The correlation between the functional aerobic impairment, peak arterio-venous O_2 difference and peak systemic vascular resistance suggests that impaired oxygen delivery and extraction by the periphery in transplant

recipients, contribute to the decreased exercise capacity. Increased peripheral vascular resistance could be brought about by vascular changes from long standing heart failure (Zelis and Flaim, 1982), deconditionning (Sinoway et al., 1986), and/or the effect of cyclosporine on the vasculature (Golub and Berger, 1987; Carrier et al., 1991) and could decrease blood flow to the exercising skeletal muscle. Persistence of peripheral skeletal muscle atrophy and impaired oxidative metabolism (Sullivan et al., 1990) due to irreversible changes from long-standing heart failure, deconditionning (Klaussen et al., 1981) and possibly the effect of corticosteroid on the skeletal muscle (Ruff, 1986) could contribute to the decreased a-v O_2 difference. The associated decrease in oxygen delivery and uptake by the periphery could cause an earlier onset of anaerobic metabolism than normal and increased the functional impairment. Deconditionning could also play an important role in the increased functional impairment in cardiac transplant recipients since twenty-one days of bed rest decrease the peak oxygen uptake but do not alter the cardiac output-oxygen uptake relationship in normals (Saltin et al., 1968).

In summary the present results suggest that the decreased exercise capacity in late cardiac transplant recipients is complex in origin, but factors such as peripheral vascular noncompliance and decreased oxygen extraction are important. These may be the result of irreversible changes from long standing heart disease, deconditioning, and/or the effects of medication on the vasculature and the skeletal muscles. Further studies need to evaluate the peripheral vascular compliance and skeletal muscle following cardiac transplantation, in order to enhance our understanding of their role in limiting the rehabilitation potential of the cardiac transplant recipients.

CHAPTER SIX

CARDIOPULMONARY RESPONSES IN CARDIAC TRANSPLANT RECIPIENTS ARE INFLUENCED BY POSTURE

6.1 INTRODUCTION

In normal subjects, right and left ventricular filling pressures increase only minimally during exercise, regardless of the exercise posture (Granath et al., 1964; Thadani et al., 1978). In addition, the relationship between cardiac output and oxygen uptake is linear and reproducible (Bevegard et al., 1963; Ekelund and Holmgren, 1967; Faulkner et al., 1977) and the slope of the relationship is not affected by posture (Ekelund and Holmgren, 1967). In cardiac transplant recipients, a marked elevation of both right and left ventricular filling pressures has been documented during supine exercise (Campeau et al., 1970; Hosenpud et al., 1989a, Pflugfelder et al., 1988), whereas the responses during upright exercise are comparable to normal (Rudas et al., 1990). Using the rebreathing technique to measure cardiac output, Jensen et al. (1991) and Kavanagh et al. (1988) found a near normal relationship between cardiac output and oxygen uptake in cardiac transplant recipients during upright graded exercise. The relationship between cardiac output and oxygen uptake in the supine posture has not been documented.

The purpose of the present study was to test the hypothesis that the apparent abnormalities in cardiac performance noted during supine exercise in cardiac transplant recipients adversely affects exercise capacity and in particular the cardiac output-oxygen uptake relationship.

6.1 METHODS

6.2.1 Patients

Thirty eight male and 2 female cardiac transplant recipients aged 51±2 years (range 24 to 62) were studied at the time of their routine evaluation three months to nine years post-transplantation. Twenty patients underwent upright exercise testing and 20 underwent supine exercise testing with concomitant right sided hemodynamic measurements and mixed expired gas analysis. There were no significant differences in patient characteristics between the two groups (Table 12). All patients were considered fully rehabilitated and had no evidence of rejection that required modification of their immunosuppressive therapy at the time of study. Patients with severe graft coronary artery disease, defined as more than 30% stenosis of a major epicardial vessel or diffuse small vessel disease on coronary angiography, were not included. Immunosuppressive therapy at the time of testing consisted of prednisone and cyclosporine for all patients. In 22 patients, azathioprine was added. Thirty patients were being treated for hypertension at the time of study, 15 in each group. Six patients were receiving an angiotensin inhibitor (two in the upright exercise group and four in the supine exercise group), 18 patients were treated with a calcium channel blocker (nine in the upright exercise group and nine in the supine exercise group) and 10 were receiving a diuretic (six in the upright exercise group and four in the supine exercise group).

6.2.2 Exercise protocol

After a routine endomyocardial biopsy, performed through the right internal jugular vein, a flow directed thermodilution catheter was introduced and advanced into a branch of the right pulmonary artery for hemodynamic measurements at rest and during exercise. Graded upright or supine bicycle exercise was performed to a symptom limited maximum. The initial workload

was 25 Watts increasing by 25 Watts every 3 minutes to the point of fatigue. Central hemodynamics were measured at rest in the supine position, at rest in the exercise posture (legs elevated on the cycle pedals in the supine exercise group and sitting on the cycle ergometer in the upright exercise group), and at the end of each exercise stage as previously described (Rudas et al., 1990). At two minutes into each exercise stage, central hemodynamics were measured in the following sequence: pulmonary artery phasic and mean pressures, mean pulmonary capillary wedge pressure, and mean right atrial pressure. Thermodilution cardiac outputs were measured in duplicate or triplicate at the end of each exercise stage to obtain values in agreement by 10%. Arterial blood pressure was measured using an automated sphygmomanometer at rest and toward the end of each exercise stage.

Mixed expired gases were sampled continuously during the exercise test (rate 4-6 L/min) and were analyzed for O_2 and CO_2 content. Data were processed on line by a microcomputer programmed to correct for delays between signals for volume and gas concentration and presented the average values during every 10-15 second interval for the following variables: minute ventilation, tidal volume, respiratory rate, oxygen uptake, carbon dioxide output, respiratory exchange ratio and heart rate. The ventilatory threshold was determined by the V-slope method (Beaver et al., 1986).

In addition, to further evaluate the effect of posture on the cardiopulmonary response to exercise, 20 patients mostly from the supine group underwent a second exercise test in the other posture within one week of the first exercise test. The exercise protocol was identical to the first except that right heart hemodynamic measurements were not obtained.

6.2.3 Statistical analyses

Peak exercise central hemodynamics were defined as those values measured in the last completed exercise stage. Peak heart rate and peak oxygen uptake were those recorded at peak exercise. Data are expressed as means ± SEM. Means were compared by the two-tailed Student's t-test for unpaired or paired samples where applicable. Individual cardiac output-oxygen uptake relationships were obtained from standard linear regression analysis using the least squares method. The mean of individual slopes and intercepts in each group were compared by unpaired t-testing. A p value <0.05 was considered statistically significant.

6.3 RESULTS

Hemodynamic responses to exercise. At rest in the supine position, right atrial pressures were similar in the two groups. At submaximal and maximal exercise, right atrial pressures were significantly higher in the supine position than the upright position (Figure 14A). Similarly, resting pulmonary capillary wedge pressures were not different in the two groups at rest (Figure 14B) but at submaximal and maximal exercise the wedge pressure was significantly higher in the supine position.

Cardiopulmonary responses to exercise. In each individual subject, the cardiac output-oxygen uptake relationship was linear with an r value ≥ 0.88. When individual regression lines were compared, a significant difference in the slope of the cardiac output-oxygen uptake relationship was observed between postures. Cardiac transplant recipients exercising in the supine position had a lower increase in cardiac output for a given oxygen uptake than recipients exercising in the upright posture. The mean slope and intercept of the regression lines relating cardiac output to VO_2 in the supine position were

4.60±0.28 and 5.22±0.25, compared to 5.95±0.28 (unpaired t-test, $p<0.005$ versus supine) and 3.37±0.17 (unpaired t-test, $p<0.005$ versus supine) in the upright posture. Using multiple regression analysis, on all data points, the effect of posture on the cardiac output-oxygen uptake relationship was also significant ($p<0.001$).

In the 20 patients who underwent both supine and upright bicycle exercise testing, peak oxygen uptake, peak heart rate, and ventilatory threshold were significantly lower in the supine position, by 16%, 7%, 21% respectively (Table 13).

Table 12. Characteristics of cardiac transplant patients in the upright or supine exercise group

	Supine exercise n=20	Upright exercise n=20
Gender (M/F)	19/1	19/1
Time post transplant (yrs)	2.7±0.3	2.1±0.4
Recipient age (yrs)	52±2	50±10
Height (m)	1.78±0.01	1.75±0.06
Weight (kg)	82±3	76±12
Donor age (yrs)	26±2	27±9
Ischemic time (min)	205±24	209±21
Hemoglobin (gm/L)	133±3	132±2

Table 13. Cardiopulmonary exercise test results for 20 patients exercised in both the upright and supine positions

	Supine exercise n=20	Upright exercise n=20
HR max (beats/min)	137±4	147±5
Vo_2 max (ml/kg/min)	19.4±1.2	22.6±1.7*
VT (ml/kg/min)	10.2±0.4	12.3±0.8*
Work time (sec)	643±53	715±68*

HR=heart rate; Vo_2 max= peak oxygen uptake; VT= ventilatory threshold

* $p<0.02$ vs supine

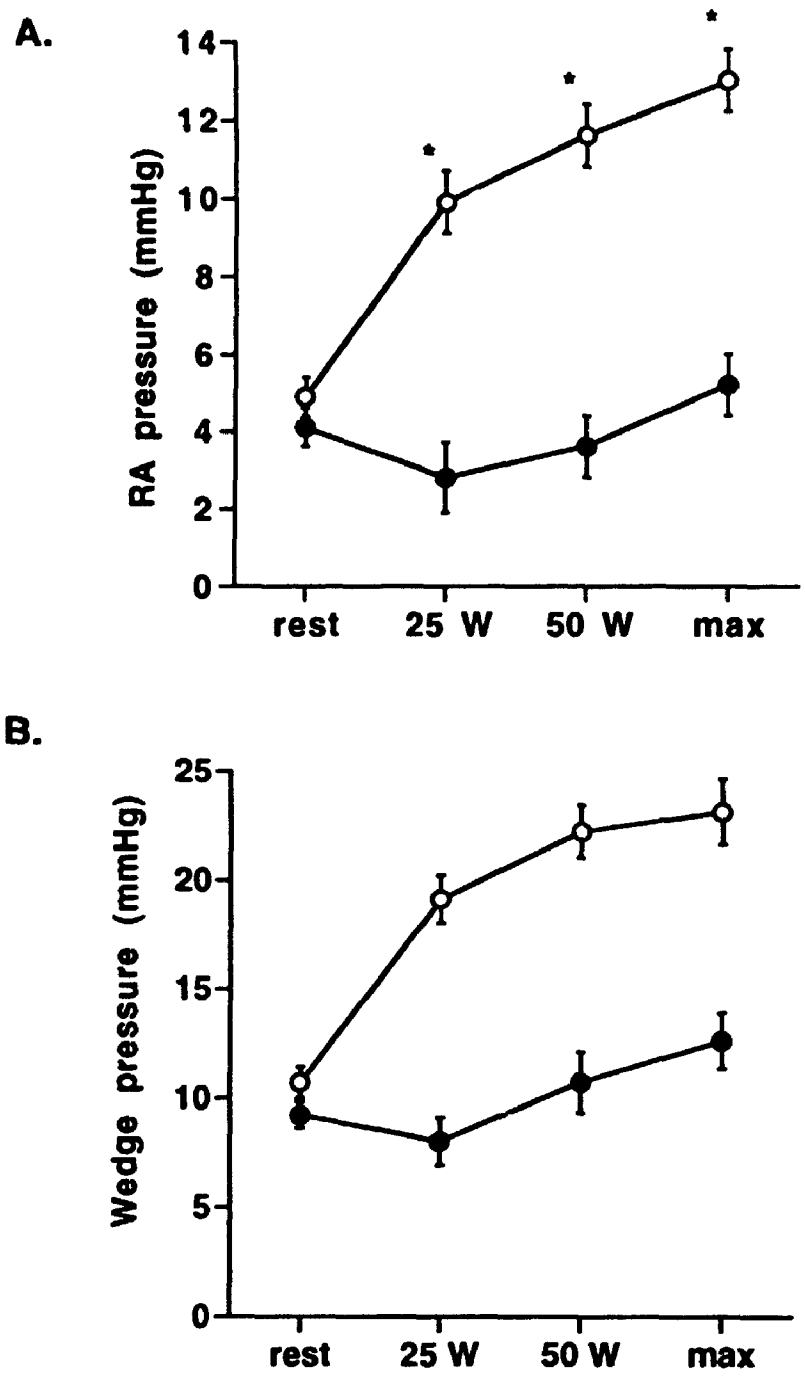


Figure 14. Right atrial (A) and pulmonary capillary wedge pressures (B) at rest and during supine (open circle) and upright (closed circle) cycle exercise in cardiac transplant patients. Values are means±SEM. * Significantly different from upright ($p<0.05$).

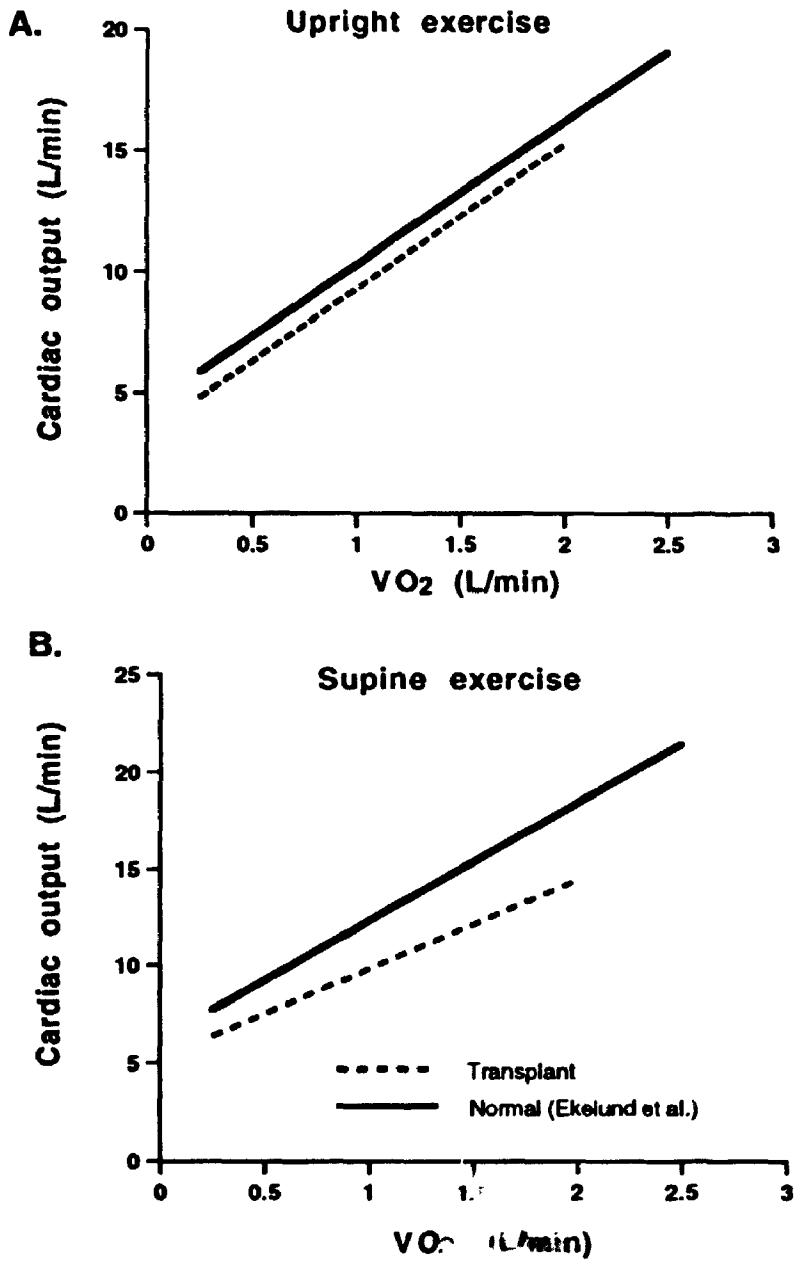


Figure 15. Relationship between oxygen uptake and cardiac output during upright (A) and supine (B) exercise in cardiac transplant recipients compared to the reported normal response (Ekelund and Holmgren, 1967).

6.4 DISCUSSION

The present study demonstrates that cardiopulmonary responses to exercise in cardiac transplant recipients are influenced by body position. Exercise in the supine posture resuits in markedly higher central pressures, lower cardiac output augmentation for a given increase in oxygen uptake, lower peak oxygen uptake, and lower ventilatory threshold than exercise in the upright posture. These results provide further evidence for impaired function of the transplanted heart, unmasked only by volume loading (Humen et al., 1984; Young et al., 1987) or exercise in the supine posture (Hosenpud et al., 1989a; Pflugfelder et al., 1988; Rudas et al., 1990).

Although there continues to be controversy as to whether the cardiac output-oxygen uptake relationship is linear or curvilinear, most studies agree that the relationship in healthy subjects is linear and that the slope is quite reproducible, approximating 5 to 6 litres/min per litre of oxygen uptake (Bevegard et al., 1963; Ekelund et al., 1967; Faulkner et al., 1977). Our data indicate that the relationship in transplant recipients fits the linear model with an r value in excess of 0.85 in all patients. In normal subjects, body posture has no effect on the slope of the relationship but a lower cardiac output (2 litres) is maintained throughout exercise in the upright posture (Bevegard et al., 1963; Ekelund and Holmgren, 1967). The downward shift of the relationship between cardiac output and oxygen uptake during upright exercise appears to be due in part to central and peripheral circulatory adaptations of blood volume and cardiac output distribution (Folkow et al., 1971; Reeves et al., 1961).

The effect of body posture on the relationship between cardiac output and oxygen uptake has not been investigated in cardiac transplant recipients. Using the rebreathing technique to measure cardiac output, Jensen et al. (1991) and Kavanagh et al. (1988) have demonstrated that the relationship

between cardiac output and oxygen uptake during upright exercise is comparable to that of normal subjects. The current study confirms these results using direct measurements of cardiac output. The slope of the relationship between cardiac output and oxygen uptake during upright cycle exercise was 5.95 litres per litre of oxygen uptake, similar to normal (Figure 15A).

Less is known about the relationship between cardiac output and oxygen uptake during supine exercise in cardiac transplant recipients. Prior to the introduction of cyclosporine, Griepp et al. (1971) demonstrated that cardiac transplant recipients had a lower cardiac output for a given oxygen uptake during supine exercise when compared to normal and postulated that the lower cardiac output was due to a downward displacement of the slope of the relationship resulting from a lower resting cardiac output. In the current study, however, we have observed a lower slope of the relationship of cardiac output and oxygen uptake compared to recipients exercising in the upright posture and the reported normal supine response (Figure 15B), rather than simply a downward displacement of the slope. The discrepancy in the studies can in part be explained by the higher resting cardiac output observed in our patients which was well within the normal range.

In the 20 patients who underwent both supine and upright bicycle exercise testing, peak heart rate and oxygen uptake were significantly lower and the ventilatory threshold occurred at a significantly lower oxygen uptake in the supine position (Table 13), suggesting impaired cardiac performance may be unmasked by exercise in this posture. A number of factors such as injury at the time of harvesting (Burt and Copeland, 1986), cardiac denervation, post-operative allograft rejection and fibrosis (Pickering and Boughner, 1990) can potentially contribute to impaired cardiac performance in cardiac transplant recipients. The ensuing diastolic dysfunction (Hausdorf et al., 1989; Humen et

al., 1984; Young et al., 1987) become more apparent in the setting of higher baseline ventricular end-diastolic volumes, i.e. supine posture and leg elevation (Hosenpud et al., 1989a; Pflugfelder et al., 1988). It is also possible, however, that the abnormal relationship between cardiac output and oxygen uptake might be the result of altered peripheral circulatory and/or sympathetic nervous system responses to supine exercise in cardiac transplant recipients as a consequence of denervation, long standing congestive heart failure or immunosuppressive therapy. Cyclosporine increases sympathetic activation (Scherrer et al., 1990) and causes peripheral vasoconstriction (Golub and Berger, 1987).

In summary, the current results suggest that cardiac transplant recipients are able to maintain their oxygen carrying capacity within the normal range during upright exercise. During supine exercise, however, oxygen transport is limited as demonstrated by the lower cardiac-output oxygen uptake relationship, peak oxygen uptake and ventilatory threshold, further supporting subtle impairment of function of the transplanted human heart.

CHAPTER SEVEN

EFFECTS OF INCREASED FILLING PRESSURE ON THE RELEASE OF ATRIAL NATRIURETIC PEPTIDE DURING EXERCISE IN CARDIAC TRANSPLANT RECIPIENTS

7.1 INTRODUCTION

Atrial natriuretic peptide (ANP) is an important hormone in the regulation of sodium and water balance (De Bold et al., 1981) and has potent vascular relaxant properties (Currie et al., 1983). A number of animal (Edwards et al., 1988; Ledsome et al., 1985) and human (Bates et al., 1986; Sato et al., 1986; Rodeheffer et al., 1986) studies have demonstrated that atrial distention is associated with increased ANP levels in the innervated heart. Similarly, during dynamic exercise, plasma ANP increases in normal subjects in proportion to increases in left ventricular filling pressures (Kitzman et al., 1989). Less is known, however, about the stimulus for ANP release in the denervated human heart, e.g. in cardiac transplantation. Despite near normal filling pressures at rest, cardiac transplant recipients have markedly elevated plasma ANP levels (Deray et al., 1990; Dussaule et al., 1990; Magovern et al., 1987; Singer et al., 1986). A marked difference in the hemodynamic responses of cardiac transplant recipients during supine and upright exercise has been observed by Rudas et al. (1990). Marked elevation of both right and left ventricular filling pressures occurs during supine exercise whereas responses during upright exercise are normal. In light of these findings, we have investigated the effects of exercise in the upright and supine positions, on the changes in central

hemodynamics and plasma levels of immunoreactive ANP (irANP), in 24 cardiac transplant recipients.

7.2 METHODS

7.1.1 Patient population

Twenty-four male cardiac transplant recipients aged 22 to 60 years (mean 47±2 years) underwent either a supine (n=12) or upright (n=12) exercise test three months to seven years post transplantation. Patient characteristics are listed in Table 14 and were similar in both groups except for the time post transplantation which was greater for the supine group. The immunosuppressive regimen consisted of prednisone and cyclosporine. Azathioprine was added to this regimen in six patients in the upright and 10 patients in the supine group. In addition, 10 patients in each group were receiving either one or two of the following antihypertensive agents; calcium channel blockers (five patients upright and seven supine), diuretics (six patients upright and two supine) or an angiotensin converting enzyme inhibitors (one patient upright and two supine).

7.2.2 Exercise test protocol

After a routine endomyocardial biopsy performed through the right internal jugular vein, a flow directed thermodilution catheter was advanced into a branch of the right pulmonary artery for hemodynamic measurements at rest and during exercise. The pressure transducer was positioned at the level of the midaxillary line when the patient was lying supine and at the level of the fourth intercostal space on the right when sitting on the bicycle for upright exercise. The exercise test consisted of graded upright or supine bicycle exercise performed to a symptom limited maximum. The initial workload was 25 Watts increasing by 25 Watts every 3 minute until the point of fatigue. Central

hemodynamics were measured at rest in the supine position, at rest in the exercise position (legs elevated in the cycle pedals in the supine exercise group and sitting on the cycle ergometer in the upright exercise group), and at the end of each exercise stage as previously described for our laboratory (Rudas et al., 1990). Blood was drawn from the right atrium for the ANP determination at rest, in the supine position, the exercise position, and at peak exercise. Mixed expired gases were analyzed continuously during the exercise test via O_2 and CO_2 analyzers (Ametek).

7.2.3 ANP determination

Blood samples for ANP analysis were collected in prechilled tubes containing aprotinin (Trasylol). Sample tubes were placed on ice and then centrifuged at 4°C. Plasma was separated and stored at -20°C until analysis. Plasma levels of ANP were measured by radioimmunoassay using a commercially available kit (INSTAR Corporation, Stillwater, Minnesota). Plasma extraction was performed using immunoextraction and ANP levels determined using sheep anti-ANP serum.

7.2.4 Statistical analyses

Results are presented as means ± SEM. Resting and peak exercise values within the same groups were compared using a two-tailed paired t-test whereas comparison between the two groups was done using the unpaired t-tests. Correlations between the change in irANP and hemodynamic responses to exercise were obtained by linear regression. A p value of less than 0.05 was considered significant.

7.3 RESULTS

Resting supine central hemodynamics were similar in both groups (Figure 16). However when patients assumed the resting exercise position central hemodynamic changes responded in opposite directions. Both the right atrial and pulmonary capiliary wedge pressures fell upon assuming the upright posture sitting on the bike, whereas in the supine posture the central pressures tended to increase with leg elevation. Central pressures increased in both groups with exercise but, patients exercising in the supine posture had a much greater increase in right atrial and pulmonary capillary wedge pressures. At peak exercise, the right atrial and pulmonary capillary wedge pressures in the supine posture were almost twice the values observed in the upright group (Table 15, Figure 16). Peak oxygen uptake, heart rate, and blood pressure were similar in both groups (Table 15).

Plasma irANP levels were comparable at rest in the supine posture in both groups (Figure 17). Non significant correlations were observed between resting supine irANP and resting central hemodynamics, creatinine level, donor age and total ischemic time. With exercise, ANP levels increased significantly in both groups. However, the average maximal irANP level in patients exercising in the supine posture was 51 % greater than the value observed during the upright posture. To determine if the increase in plasma irANP paralleled the increase in central hemodynamics, correlations were performed between increase in irANP levels and the changes in hemodynamic measurements from rest to peak exercise. The increase in irANP levels correlated significantly with the changes in right atrial, systolic pulmonary artery, and pulmonary capillary wedge pressures but not with heart rate, mean systolic blood pressure or peak oxygen uptake (Table 16).

Table 14. Characteristics of cardiac transplant patients undergoing upright or supine exercise

	Upright exercise n=12	Supine exercise n=12
Pre transplant Diagnosis		
Coronary artery disease	8	7
Cardiomyopathy	4	5
Age (yrs)	44±4	51±3
Height (m)	1.76±0.02	1.78±0.02
Weight (kg)	79±4	80±3
Hemoglobin (g/L)	132±5	133±5
Creatinine (umol/L)	133±8	147±9
Donor age (yrs)	26±3	21±1
Total ischemic time (min)	237±83	246±31
Time post transplantation (yrs)	1.0±0.3	2.7±0.6*

Means±SEM; * p<0 05 vs upright

Table 15. Cardiopulmonary and central hemodynamic measurements at peak exercise in cardiac transplant recipients exercised in the upright or supine position

	Upright exercise n=12	Supine exercise n=12
Peak oxygen uptake (ml/kg/min)	23.6±1.8	20.1±1.8
Respiratory exchange ratio	1.17±0.03	1.12±0.03
Heart rate (beats/min)	144±4	143±5
Systolic blood pressure (mmHg)	185±6	189±5
Diastolic blood pressure (mmHg)	96±5	100±2
Right atrial pressure (mmHg)	7±1	12±1†
Pulmonary capillary wedge pressure (mmHg)	14±2	22±6†
Cardiac index (L/min/m^2)	6.6±0.5	6.6±0.4
Pulmonary vascular resistance (mmHg/L/min)	1.4±0.2	1.0±0.1*

Means±SEM; *p<0.05 vs upright; †p<0.005 vs upright

Table 16. Correlation coefficients between the change in atrial natriuretic peptide (max-rest) and the changes in central hemodynamic and cardiopulmonary parameters during exercise

Parameters	r	p
VO_2 max	0.15	0.52
Δ HR	0.37	0.095
Δ BP mean	0.35	0.17
Δ RAP	0.50	0.020*
Δ PCWP	0.60	0.0035*
Δ PAP sys	0.79	0.00020*
Δ CI	0.37	0.095
Δ SVR	-0.21	0.46
Δ PVR	-0.26	0.26

CI=cardiac index; HR=heart rate; MBP=mean blood pressure; PAPsys=systolic pulmonary artery pressure; PCWP=pulmonary capillary wedge pressure; PVR=pulmonary vascular resistance; RAP=right atrial pressure; SVR=systemic vascular resistance; VO_2 max=peak oxygen uptake

* $p<0.05$

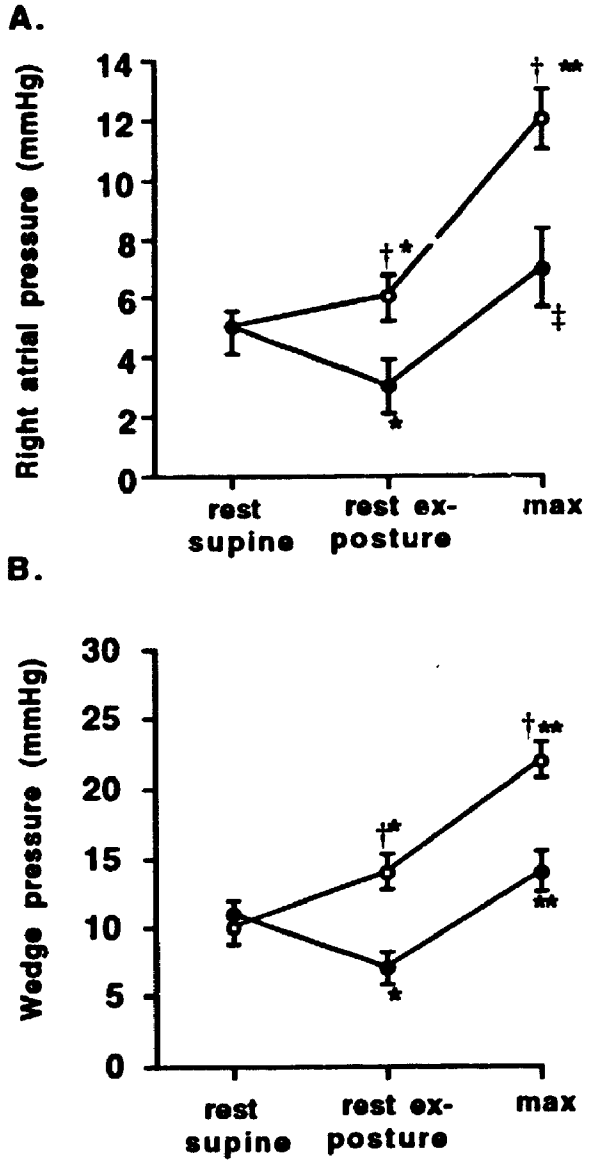


Figure 16. Right atrial (A) and pulmonary capillary wedge pressures (B) at rest and during supine (open circles) and upright (closed circles) cycle exercise in cardiac transplant patients. Values are means±SEM. † Significantly different from upright ($p<0.05$); * Significantly different from rest supine ($p<0.05$); ** Significantly different from rest supine and rest position ($p<0.05$). ‡ Significantly different from rest position ($p<0.05$).

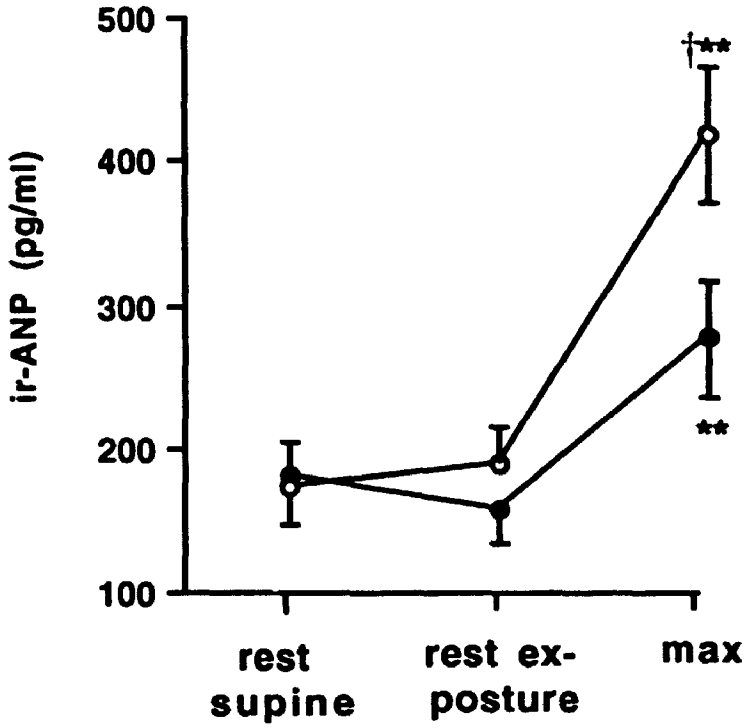


Figure 17. Immunoreactive atrial natriuretic peptide at rest and during supine (open circles) and upright (closed circles) cycle exercise in cardiac transplant patients. Values are means±SEM. † Significantly different from upright ($p<0.05$); * Significantly different from rest supine ($p<0.05$); ** Significantly different from rest supine and rest position ($p<0.05$).

7.4 DISCUSSION

Right and left atrial distention has been reported to be an important stimulus for the release of atrial natriuretic peptide in normal subjects (Kenneth, 1988). In cardiac transplant recipients, however, elevated irANP levels occur despite near normal resting filling pressures (Deray et al., 1990; Dussaule et al., 1990; Magovern et al., 1987; Singer et al., 1986). The mechanism underlying the increased plasma atrial natriuretic peptide concentration in transplant recipients is not clear but factors such as altered atrial anatomy (15), recipient versus donor atrial dysynchrony, increased atrial size (Dussaule et al., 1990), decreased irANP clearance due to renal insufficiency related to cyclosporine treatment (Myers et al., 1984) and cardiac denervation have been implicated. In addition, increased sympathetic activation noted in cardiac transplant recipients (Scherrer et al., 1990) could also be an important factor as there is evidence that the sympathetic nervous system participates in the release of ANP (Kuchel et al., 1987).

The physiological consequence of the increased level of irANP in cardiac transplant recipients is unknown. The presence of hypertension in up to 70% of long term cardiac transplant recipients using cyclosporine (Thompson et al., 1983) suggests that receptor down regulation due to the high level of circulating ANP from previous long standing heart failure (Cody et al., 1986; Raine et al., 1986) may persist after cardiac transplantation or that increased sympathetic vasoconstrictor tone may overrides the effects of ANP. In the present study, cardiac transplant recipients demonstrated elevated levels of plasma irANP when compared to the laboratory controls (176±24 vs normal 0-40 pg/ml). Similar levels have been observed by others in cardiac transplant recipients (Deray et al., 1990; Dussaule et al., 1990; Magovern et al., 1987). No significant correlations were found between resting levels of irANP and right or left atrial

pressures. This is in agreement with the findings of Dussaule et al. (1990) and Deray et al. (1990) but in contrast to those of Magovern et al. (1987). These conflicting results suggest the exact mechanism for increased in irANP at rest in cardiac transplant recipients remains unknown.

Despite the elevated levels observed at rest, cardiac transplant recipients have a marked increase in irANP during exercise. Previous studies on the plasma irANP response to exercise in cardiac transplant patients, have reported increased irANP levels of 110 (Mettauer et al., 1991) and 230% (Starling et al., 1991) during supine exercise and 100 (Pepke-Zaba et al, 1992) and 150 % (Singer et al., 1990) during upright exercise. In the current study similar increases in plasma irANP were observed during exercise. However the average increases in plasma irANP of 76 % during upright exercise was significantly lower than the 123 % increases observed during supine exercise. These results are in contrast to the normal response of subjects exercising in the supine and upright position where irANP concentrations at peak exercise are virtually identical (Perrault et al., 1989). These results further support the importance of filling pressures as a stimulus for irANP release during exercise as in normal subjects, unlike cardiac transplant recipients the ventricular filling pressures increases to much the same extent during either upright or supine exercise (Thadani et al., 1978).

The present study provides unique insight into the stimulus for the increase in ANP during exercise in cardiac transplant recipients. The marked difference in the central filling pressures in response to exercise in the supine and upright postures allowed the effect of variables (heart rate, blood pressure, cardiac output) which also increase during exercise to be eliminated. Increases in heart rate, blood pressure, cardiac index and oxygen uptake did not correlate with the increased irANP levels. While a decrease in metabolic clearance could

cause a rise in ANP levels during exercise, the increased plasma levels appeared to be the result of an increased secretion from cardiac tissue brought about by changes in central filling pressure. Supporting this hypothesis are the strong correlations observed between the changes in right atrial, systolic pulmonary artery and pulmonary capillary wedge pressures and the increase in irANP. The source of the circulating irANP in cardiac transplant recipients is uncertain. The donor atria, the recipient atrial remnants and/or the ventricular myocytes may all serve as sources for irANP. Ventricular expression of irANP has been reported in conditions where the ventricle is overstretched (Gu et al., 1991). The strong correlation between ANP levels and systolic pulmonary artery pressure might suggest a secretory role by the right ventricle.

One of the limitations of the present study is that two groups of patients were compared. While most of the patients' characteristics were similar, patients exercised in the upright posture were studied earlier after transplantation than patients exercised in the supine posture. However it is unlikely that the difference in the time post transplantation could explain the large difference in irANP observed at peak exercise. Previous studies have noted a small decrease of plasma ANP on the first day after transplantation but ANP returned to pre-transplant levels thereafter (Mertes et al., 1991). In the present study, we have not observed any relationship between the resting ANP levels and the time post transplantation. Similarly no relationship was found between the peak levels or the increase in plasma ANP during exercise and the time post transplantation .

In summary, in cardiac transplant recipients, exercise is a stimulus for ANP secretion and augmentation in plasma irANP levels during exercise appears to be modulated by changes in central hemodynamics. In the present study strong correlations were observed between the increased irANP levels

and the changes in right atrial, systolic pulmonary artery pressure and pulmonary capillary wedge pressure. The exact source of the circulating irANP in cardiac transplant recipients, however, remains uncertain.

CHAPTER EIGHT

EXERCISE RESPONSES AFTER CARDIAC TRANSPLANTATION IN MITOCHONDRIAL MYOPATHY

8.1 INTRODUCTION

Since the first description by Luft et al in 1962, mitochondrial myopathy has become an increasingly recognized cause of neuromuscular disease. A wide variety of clinical syndromes has been described in patients with these disorders (Morgan-Hughes, 1986). Although many clinical presentations appear to be restricted to skeletal muscle, other highly oxidative organs including the central nervous system, kidney, liver and heart have been affected in some patients with multisystem mitochondrial abnormalities. In the heart, cardiac conduction defects have been described in many patients with mitochondrial myopathy (Karpati et al., 1973; Olson et al., 1972), whereas cardiomyopathy has been reported infrequently (Bender and Engel, 1976; Hubner et al., 1986; Neustein et al., 1979; Sengers et al., 1976; van Ekeren et al., 1987; Zeviani et al., 1991). In this report we describe the morphologic findings of cardiac and skeletal muscle and the unique cardiorespiratory exercise responses in two patients who underwent successful cardiac transplantation for end-stage cardiomyopathy resulting from mitochondrial myopathy.

8.2 METHODS

8.2.1 Patient population

Patient One. A 23-year-old man with a two-year history of dilated cardiomyopathy and heart failure presented to hospital with a one-week history of nausea and vomiting followed by rapid deterioration in clinical status. His past medical history was unremarkable except for a history of exercise intolerance since childhood. There was no history of neuromuscular disease in the family. At the time of transfer to our institution for consideration of cardiac transplantation, he was critically ill requiring intraaortic balloon counterpulsation and large doses of inotropic agents to maintain marginal tissue perfusion. Three days after transfer, a 47 year old male donor became available and the patient underwent orthotopic cardiac transplantation. The post-operative course was uneventful apart from prolonged jaundice which resolved spontaneously. Within the second post operative week the patient was ambulatory and started a program of physiotherapy.

Patient Two. A 33-year-old man with a five-year history of dilated cardiomyopathy presented to our institution with a history of progressive heart failure (New York Heart Association class III to IV) for several months. He had required a pacemaker two years previously for third degree atrioventricular block. There was a 10-year history of central nervous system impairment including ataxia, reduced vision with retinal atrophy, seizures and decreased auditory acuity. However, he remained employed full time until a short time before assessment. The patient's mother had died at a young age of cardiomyopathy and what was thought to be multiple sclerosis. An older brother has severe central nervous system disease but no clinical evidence of cardiac involvement. At the time of initial evaluation, neurological symptoms were marked, but because he was more incapacitated by his cardiac disease it

was believed that he would benefit from cardiac transplantation. Two months after his initial assessment a 38-year-old male donor became available and the patient underwent orthotopic cardiac transplantation. The early postoperative course was complicated by renal as well as liver dysfunction, which slowly resolved. Because of the long period of debility before transplantation his recovery was slow; however, there was a marked improvement in overall functional capacity.

8.2.2 Heart and skeletal muscle analyses

The explanted hearts of the patients were examined by light and electron microscopy. A skeletal muscle biopsy was performed on the vastus lateralis muscle, using the technique of Bergstrom (1962) at six months for patient one and at 12 months for patient two. Tissue samples were examined for light and electron microscopy.

8.2.3 Exercise test protocol

Exercise testing consisted of a graded upright bicycle test performed to a symptom-limited maximum with concomitant hemodynamic and mixed expired gas measurements. Mixed venous blood was drawn from the right atrium for lactate and catecholamine determinations at rest, during exercise and at peak exercise. Patients one and two were exercised at six and 12 months after transplantation, respectively.

8.2.4 Analyses

The cardiopulmonary and metabolic responses to exercise of patients one and two were compared with those of 16 cardiac transplant recipients tested within the first year after transplantation mean age 44±3 years.

8.3 RESULTS

Native heart and skeletal muscle pathology. Microscopic examination of the native heart demonstrated myocyte hypertrophy with fibrosis, patchy in patient one and more diffuse in patient two. In both patients electron microscopy revealed abnormal mitochondria. In patient one the mitochondria had poorly defined cristae and inner lucencies (Plate 1). Patient two had mitochondria with a dense lamellar pattern (Plate 1).

On light microscopy, skeletal muscle biopsy specimen contained no inflammatory infiltrates or abnormal accumulation of intracellular lipid or glycogen by Sudan red and periodic acid-Schiff reaction stains. The myosin adenosine triphosphatase stain showed a predominance of type II fibres in patient one. Patient two did not have a fibre predominance. The modified Gomori trichrome stain demonstrated a few subsarcolemmal aggregations of red staining in patient one. Patient two had abundant red staining and granular material was seen in the subsarcolemmal area within several fibres. By electron microscopy, the mitochondria of patient one were increased in number, of variable size and occasionally of unusual shape. Most of the mitochondria had abnormal and convoluted cristae and some had dense lamellar patterns of cristae, often described as "parking lot" (Morgan-Hughes, 1986) paracrystalline inclusions (Plate 2). Patient two had large aggregates of subsarcolemmal mitochondria, many with parking lot paracrystalline inclusions (Plate 2).

Cardiopulmonary Exercise Testing. Peak oxygen uptake and the ventilatory threshold occurred at a lower oxygen uptake in both patients than in the general transplant group (Table 17). During exercise, cardiac output increased excessively relative to the muscle metabolic rate as reflected by oxygen uptake and workload. The slope of the relation between cardiac output and oxygen uptake was twice that among other transplant recipients (Table 17).

With exercise, the calculated arterio-venous oxygen difference increased minimally in patients 1 and 2 (Figure 18) compared with a doubling in the transplant group. Both patients exhibited an immediate and exaggerated increase in mixed venous lactate relative to workload and oxygen uptake (Figure 19). Similarly, both patients had a marked increase in plasma norepinephrine despite lower exercise time and capacity (Table 17).

Table 17. Results of cardiorespiratory exercise testing in two patients with mitochondrial myopathy and a group of 16 healthy cardiac transplant recipients

	P 1	P 2	Tx group
Peak workload (Watts)	100	37.5*	103±6
VO_2 max (ml/kg/m)	19.2	14.2*	22.1±1.0
VO_2 max predicted (%)	41	35	65±4
Ventilatory threshold (ml/kg/min)	9.6	7.6*	12.2±0.6
Peak heart rate (beats/min)	158	130	135±5
Peak respiratory exchange ratio	1.43*	1.46*	1.22±0.02
Peak cardiac index (L/min per m^2)	11.7*	7.6	6.5±0.3
Resting a-v O_2 difference (ml/L)	40	45	56±3
Peak a-v O_2 difference (ml/L)	51*	64*	124±5
Increase in cardiac output relative to VO_2	14.2*	10.3*	5.7±0.4
Peak lactate (mmol/L)	18.9*	8.4*	5.0±0.4
Resting plasma norepinephrine (pg/ml)	147	373	393±95
Peak plasma norepinephrine (pg/ml)	5777*	3502*	1550±166

Means±SEM; P 1=patient one; P 2= patient two; Tx group=16 healthy cardiac transplant recipients; VO_2 max= peak oxygen uptake

* >4 standard error vs normal transplant

Plate 1. Electron micrograph of myocardium from patient one (A) showing widespread mitochondrial abnormalities (disorganized, sparse, and lamellar cristae) typical in mitochondrial myopathy (x9600). Electron micrograph of myocardium from patient two (B) showing concentrically arranged cristae also typical in mitochondrial myopathy (x5600).

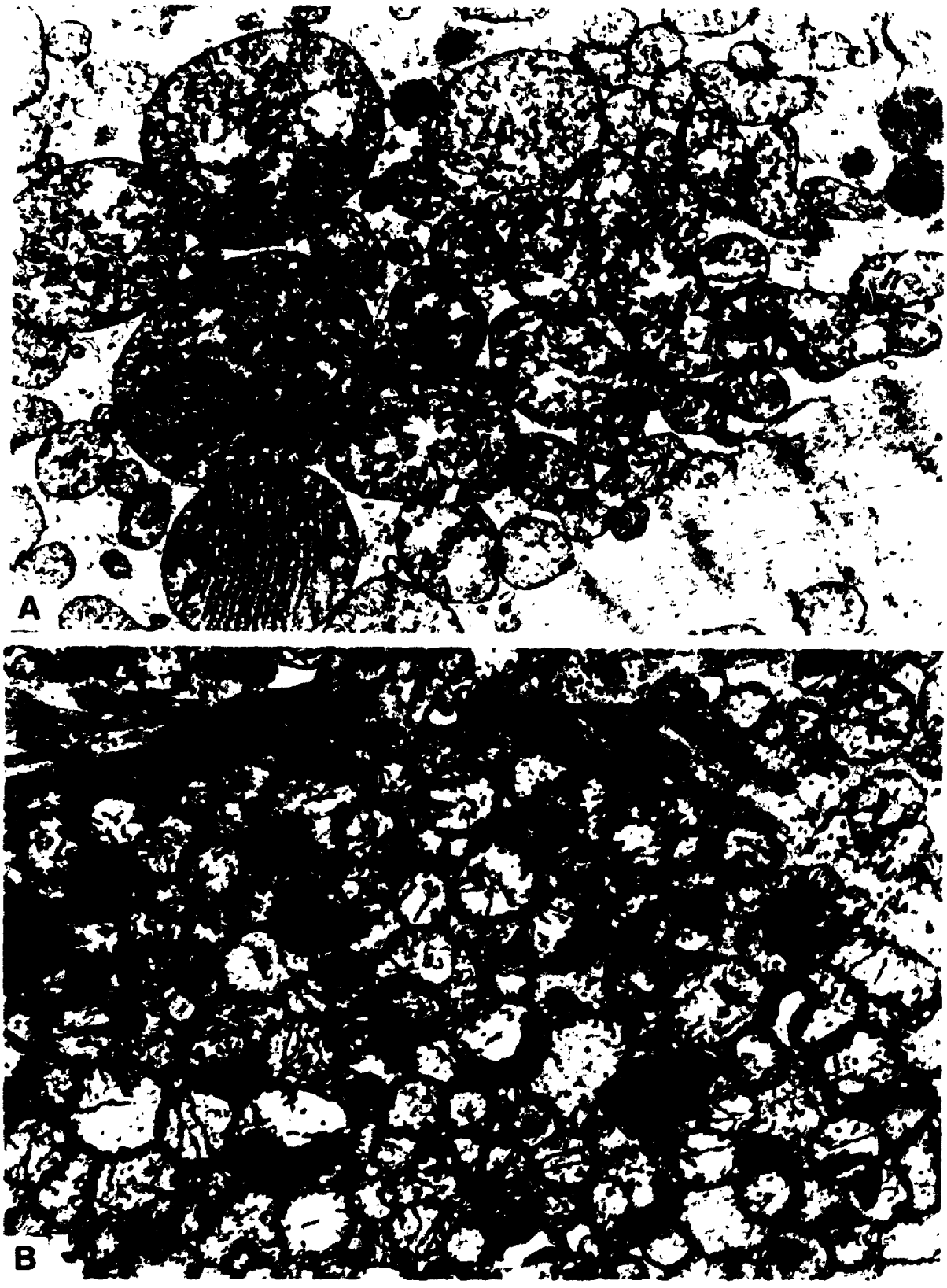

A
B

Plate 2. Electron micrograph of skeletal muscle from patient one (A) showing the typical "parking lot pattern" of paracrystalline inclusions (x16000). Electron micrograph of skeletal muscle from patient two (B) showing multiple abnormal mitochondria with paracrystalline inclusions (x24000).


A
B

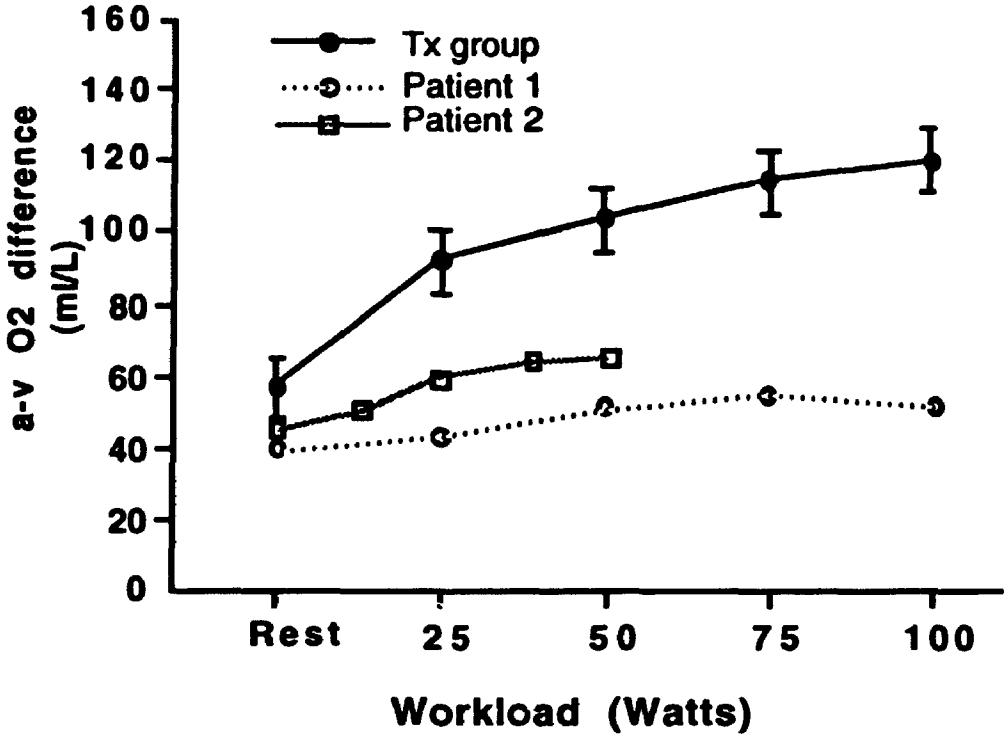


Figure 18. Relationship between systemic arterio-venous oxygen difference in ml/L and workload for patients one and two with mitochondrial myopathy compared to 16 healthy cardiac transplant recipients (Tx group). Values are means±SEM.

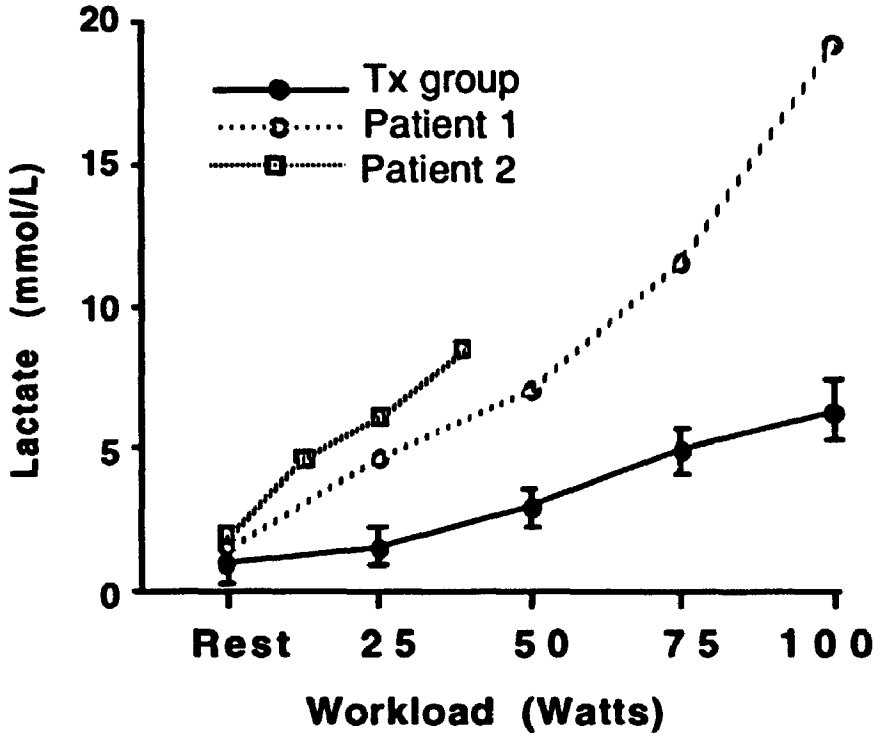


Figure 19. Relationship between plasma lactate concentration and workload in patients one and two with mitochondrial myopathy compared to 16 healthy cardiac transplant recipients (Tx group). Values are means±SEM.

8.4 DISCUSSION

The diagnosis of mitochondrial cardiomyopathy was made in these patients after cardiac transplantation when the electron microscopy of the native heart revealed the typical mitochondrial abnormalities. Skeletal muscle histology and exercise testing provided further evidence for a widespread mitochondrial disorder. To our knowledge this is the first report of patients with mitochondrial myopathy undergoing successful cardiac transplantation.

Mitochondrial myopathies are a group of disorders with clinical and biochemical heterogeneity (Harding and Holt, 1989; Morgan-Hughes, 1986). This disorder is characterized by the presence of "ragged-red" fibres in skeletal muscle biopsy tissue stained with the modified Gomori trichrome method and by ultrastructural abnormalities of mitochondria (Morgan-Hughes, 1986), notably ill-defined cristae and inclusions. Although intramitochondrial inclusions may occur in normal individuals and in other forms of myopathy (Morgan-Hughes, 1986), mitochondrial myopathy is distinguished by the presence of large collections of structurally abnormal mitochondria as the predominant abnormality in muscle fibres. Recent progress in molecular biology has enabled the characterization of specific defects of substrate oxidation in patients with these disorders (DiMauro, 1990). Impaired energy production as a result of the mitochondrial defect increases conversion of pyruvate to lactate through anaerobic glycolysis, leading to exercise intolerance with early onset of lactic acidosis (Edwards et al., 1982). Whereas severe muscle fatigability and exercise intolerance are the more common presentations, encephalopathy, cardiomyopathy, both hypertrophic (Bender and Engel, 1976; Sengers et al., 1976; Zeviani et al., 1991) and dilated (Hubner et al., 1986; Neustein et al.,

1979; Zeviani et al., 1991), retinopathy, or lactic acidosis may occasionally be the presenting feature (Morgan-Hughes, 1986).

A unique cardiopulmonary and metabolic response to exercise has been described in patients with mitochondrial disorders (Edwards et al., 1982; Haller et al., 1989). Peak oxygen uptake is generally subnormal with an exaggerated increase in lactate relative to workload and oxygen uptake. An abnormally high respiratory exchange ratio (carbon dioxide output/oxygen uptake) at peak exercise has also been described in these patients, reflecting both a decrease in oxygen uptake and an increase in carbon dioxide output from lactate buffering (Edwards et al., 1982). The respiratory exchange ratio at peak exercise of the patients presented in this report was markedly elevated when compared with patients in the transplant group and with reported normal values (Edwards et al., 1982).

The relation between oxygen transport and oxygen extraction has been well studied in patients with oxidative defects. In these patients, the increase in cardiac output during exercise is approximately two to three times normal, suggesting that blood flow is not properly coupled to local metabolic requirements (Haller et al., 1989). Correspondingly, the systemic arterio-venous oxygen difference increases minimally over resting levels, suggesting that the ability of the working muscle to extract oxygen from the circulating blood is impaired and that inadequate oxygen delivery is not the stimulus for the disproportionate increase in cardiac output. A hyperkinetic circulatory response was observed in our patients despite cardiac denervation, suggesting that the excessive cardiac output response must have been secondary to marked sympathetic activation. The high norepinephrine levels at peak exercise in these patients supports this hypothesis.

Although uncommon, mitochondrial cardiomyopathy comprised approximately two percent of patients with end-stage dilated cardiomyopathy who underwent cardiac transplantation at this institution. The long-term prognosis of these patients after cardiac transplantation is unknown. However, they have to date had a marked and sustained improvement in exercise capacity despite the atypical cardiopulmonary responses.

CHAPTER NINE

GENERAL SUMMARY AND IMPLICATIONS

The first study provided results of serial cardiopulmonary exercise testing before and after orthotopic cardiac transplantation, in 19 patients (average age 47 years) enrolled in an early rehabilitation program. The peak oxygen uptake almost doubled, from pre-transplant values, in the first three months post surgery. Before transplantation patients had blunted heart rate and blood pressure responses to exercise. Increased exercise capacity, in the first three months post transplantation, was associated with improved blood pressure and heart rate responses to exercise. The ventilatory responses to exercise also improved following transplantation. Patients awaiting transplantation had abnormal ventilatory response to exercise, with rapid shallow breathing and high minute ventilation for a given carbon dioxide output at submaximal exercise. After transplantation there was an increase in peak tidal volume and minute ventilation and a decrease in the slope of the relationship between minute ventilation and carbon dioxide output. These results suggest that increased peak oxygen uptake after cardiac transplantation is associated with significant cardiovascular as well as respiratory changes.

In the second study (Chapter Three), the changes in resting lung function after cardiac transplantation were studied. Changes in lung function have been previously described late after transplantation. However, no studies have looked at the time course of these improvements in the early post-operative period. Despite early normalization of resting central hemodynamics, improvement in most static lung function occurred over the first year post

transplantation in the present study, suggesting a slow recovery of the pulmonary function after prolong period of heart failure and pulmonary congestion. The results from Chapter Three suggest that the static abnormalities in lung function, in patients with heart failure, are in part reversible after cardiac transplantation except for the decreased diffusing capacity which persist after transplantation.

The results of the study of the changes in the peripheral skeletal muscle of 11 patients following cardiac transplantation suggest that skeletal muscle adaptations may also play a role in the improved exercise capacity following transplantation. Prior to transplantation, patients had marked atrophy in all three types of fibres. Specific skeletal muscle metabolic alterations in patients with heart failure have been previously described using 31P NMR spectroscopy. More recently using the skeletal muscle biopsy technique, atrophy of type II fibres has been observed in these patients. The uniform and pronounced fibre atrophy observed in patients awaiting transplantation probably reflects a more advanced form of the disease. After transplantation a 38 to 47 % increase in fibre cross sectional areas was observed. The activities of glycolytic and mitochondrial enzymes (expressed per gram of wet weight) also increased to a similar extent. While reversal of the skeletal muscle metabolic abnormalities has been noted in heart failure patients after exercise training using 31P NMR spectroscopy, this is the first study to demonstrate the reversibility of skeletal muscle morphology and enzymatic alterations in patients with chronic heart failure as a result of a therapeutic intervention. The increase in fibre cross sectional area, and oxidative enzyme activities after cardiac transplantation and the associated increased in peak oxygen uptake suggests that skeletal muscle adaptations may play a role in the improvement in exercise capacity after cardiac transplantation.

Despite the marked improvement in exercise capacity after cardiac transplantation, the peak oxygen uptake remained low, about 2/3 of that seen in aged matched controls. The basis for this limited exercise tolerance has not been well characterized but a list of potential factors has been put forward and includes; irreversible changes from long standing heart failure with prolonged inactivity prior to transplantation, decreased cardiac function from the effects of graft retrieval (Labovitz et al., 1991; Brubaker et al., 1993), cardiac denervation and rejection, and the repercussions of immunosuppressive drugs or other medications, post transplantation (Savin et al., 1980). In the fourth study, 57 late cardiac transplant recipients were found to have an average peak oxygen uptake equal to 66% of predicted value. In these patients peripheral factors such as impaired peripheral vasodilatation and decreased oxygen extraction at peak exercise were found to be important contributors to this suboptimal peak oxygen uptake. Total ischemic time, donor or recipient characteristics, resting cardiac function or the number of rejection episodes did not relate to the decreased exercise capacity. These results suggest that decreased exercise capacity, observed in late cardiac transplant recipients, is likely due to irreversible peripheral changes from long standing heart disease, deconditionning, and/or the effect of cyclosporine and corticosteroid on the vasculature and skeletal muscle. Cyclosporine has been shown to cause vasoconstriction in peripheral vessels whereas muscle weakness and atrophy have been reported with corticosteroid usage.

Marked increases in cardiac filling pressure have been observed in cardiac transplant patients during supine exercise whereas the response during upright exercise is comparable to normal. The fifth study determined the effects of body posture and central hemodynamics on the cardiac output and oxygen uptake relationship with exercise. The slope of the relationship was decreased

in 20 patients exercising in the supine posture when compare to 20 patients exercising in the upright position. The relationship of the upright position was comparable to that of normals as has been previously reported (Jensen et al., 1991; Kavanagh et al., 1988). The decreased oxygen transport during supine exercise in cardiac transplant recipients suggests that the diastolic dysfunction of the transplanted human heart impairs cardiac performance in the supine posture.

In the sixth study, the influence of body posture and central hemodynamics on the plasma levels of atrial natriuretic peptide (ANP) during exercise was evaluated. While increased ANP levels have been shown to be modulated by increases in atrial pressure in normal human heart, less is known about the mechanism for the release of ANP in cardiac transplant recipients. The greater increase in cardiac filling pressures during exercise in the supine posture was associated with higher plasma ANP concentrations at peak exercise when compared to that in the upright position. In addition the strong correlations observed between the changes in central filling pressures and the increases in plasma ANP during exercise, suggest that ANP levels are also modulated by changes in filling pressures, in cardiac transplant recipients.

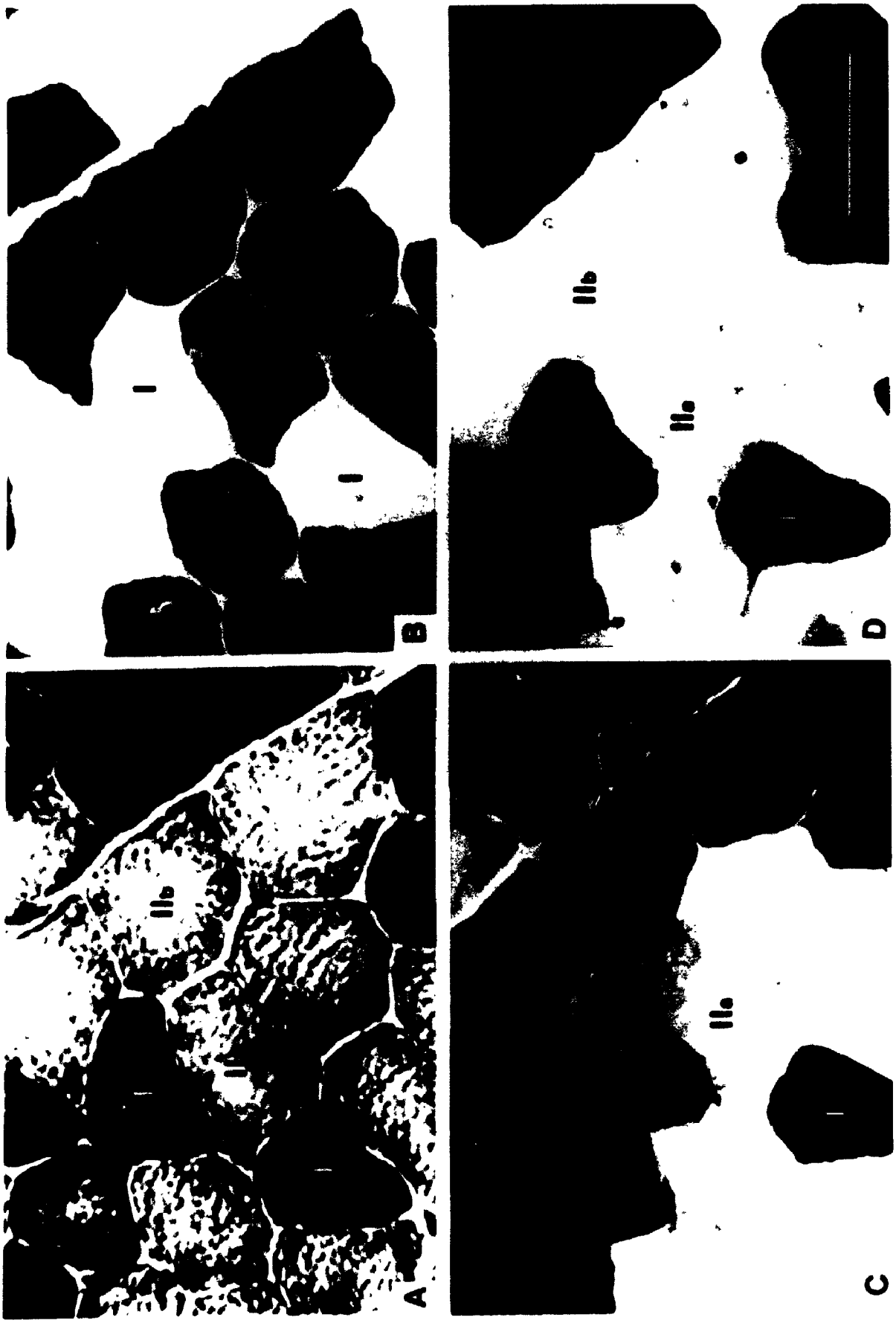
In the seventh study the exercise responses of two patients with mitochondrial myopathy, who underwent successful cardiac transplantation were evaluated. Despite denervation, these subjects demonstrated the typical cardiopulmonary and metabolic response to exercise described in patients with this disorder. They had an hyperkinetic circulatory response to exercise and the corresponding, low peak systemic arterio-venous oxygen difference in spite of earlier onset of lactate production.

The results of the data from this thesis demonstrate clearly that the improvement in exercise capacity after cardiac transplantation is complex in

origin and is not only the result of an acute improvement in cardiac function as previously thought. The improvement in peak oxygen uptake which occurred over time is apparently due to the slow reversibility of some of the changes in the cardiovascular, pulmonary and musculoskeletal systems as described in Chapters Two, Three and Four. These findings are not surprising as in normal subjects the response to physical conditioning or deconditioning involves both central and peripheral adaptations. Despite marked improvement in peak oxygen uptake after transplantation, the peak oxygen uptake remains low at about 2/3 of that predicted. While specific abnormalities in central and peripheral physiology exist in late cardiac transplant recipients, the multisystemic origin of some cardiac diseases, the irreversibility of some peripheral abnormalities that develop as a result of long standing heart failure and/or prolonged inactivity, as well as medical therapy appear to be more important factors limiting exercise capacity in the upright position. Despite suboptimal peak oxygen uptake, these patients are by no means bedridden and most of them return to normal function by 12 months post transplantation. The therapeutic efficacy of cardiac transplantation, in a subset of patients with severe end-stage heart failure, in improving and prolonging life is indisputable. The present study has provided a better understanding of the factors limiting exercise capacity before and after transplantation and offers some insight into the possible mechanisms by which exercise capacity improves after transplantation.

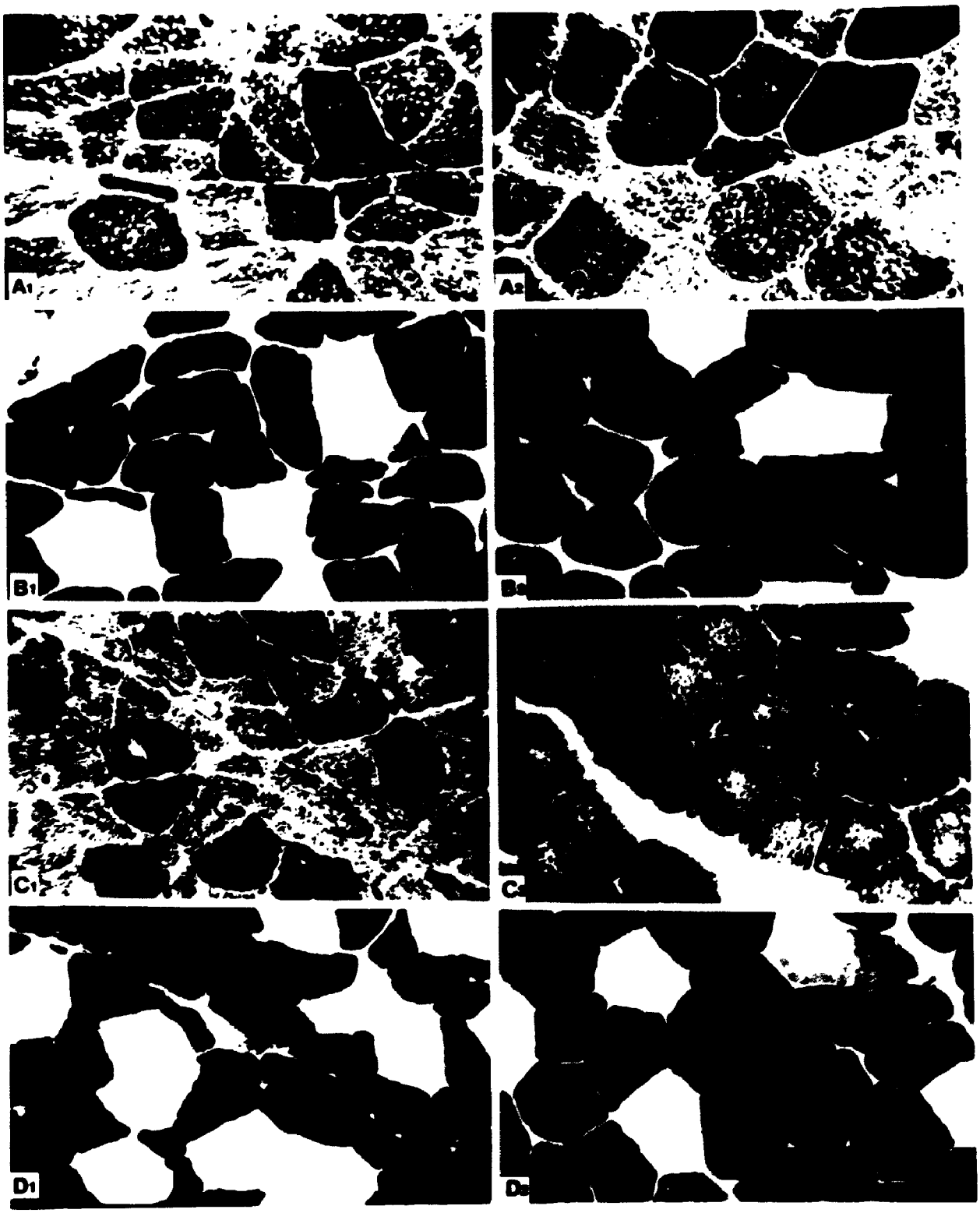
APPENDIX I

Plate 3. Photomicrographs of serial transverse sections of skeletal muscle (vastus lateralis) from a patient at 3 months following cardiac transplantation demonstrating the different staining techniques. Panel A shows a cross section stained with NADH-TR. Panels B, C and D show cross sections stained for myofibrillar ATPase with pre-incubation at pH 10.1, 4.6 and 4.3 respectively for fibre type identification. The horizontal bar in panel D denotes 100 μm.


A
B
C
D

APPENDIX II

Plate 4. Photomicrographs of serial transverse sections of skeletal muscle from two patients before (panels A1 and B1 patient one and C1 and D1 patient two) and at 12 months following cardiac transplantation (panels A2 and B2 patient one and C2 and D2 patient two). All panels are at the same magnification and the horizontal bar in panel H denotes 50 μm. Panels A1, A2, C1, and C2 show cross sections stained with NADH-TR, an increase in fibre size is noted after transplantation (panel A2 and C2). Panels B1, B2, D1, and D2 show cross sections stained for myofibrillar ATPase at pH 10.1 (dark stained fibres=Type II fibres). Panels B1 and D1 demonstrate the smaller type II fibres when compare to type I fibres in patients before transplantation. After transplantation (B2 and D2), both type I and type II fibres increased in size.


A1
A2
B1
B2
C1
C2
D1
D2

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