Altered sympathoadrenal response to dynamic exercise in cardiac transplant recipients

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ABSTRACT The cardiac denervation produced by heart transplantation modifies the physiological response to exercise. The cardiorespiratory and sympathoadrenal response of seven "healthy" orthotopic heart transplant recipients was compared to seven age matched normal subjects during progressive dynamic exercise. The initial venous noradrenaline concentration tended to be higher in the transplant group, at 3.6 (SEM 0.6) vs 2.9 (0.2) nmol·litre⁻¹ (NS). Noradrenaline concentrations were significantly higher in the transplant group during exercise (p<0.05, by analysis of variance). The transplant recipients reached a lower maximum workload than the normal subjects, at 102 (8) vs 170 (10) watts (p<0.01) and the peak noradrenaline concentrations were similar in the two groups. The fall in noradrenaline concentrations after exercise was similar in the two groups. This showed that noradrenaline clearance was normal in the transplant recipients and the higher noradrenaline level reflected increased sympathetic activity. Despite the normal peak noradrenaline concentration, the transplant recipients achieved lower maximum heart rates than the normal subjects, at 142 (3) vs 181 (5) beats·min⁻¹ (p<0.01). Adrenaline concentrations were similar in the two groups during submaximal exercise and tended to be lower in the transplant recipients at maximal exercise.

The increased sympathetic activity may be a response to altered cardiac performance because of efferent cardiac denervation or to loss of tonic inhibition of sympathetic activity by cardiac receptors due to afferent denervation. Both circulating noradrenaline and adrenaline appear to play a significant role in the heart rate response to exercise after cardiac transplantation.

The nervous system plays a key role in the control of the cardiovascular system and its adaptation to changing physiological requirements.¹ The sympathetic nervous system is of central importance in the integration of the cardiovascular response to exercise.² Sympathetic activity is altered in a variety of cardiovascular diseases and autonomic failure has profound effects on the control of the cardiovascular system.³ Cardiac transplantation is now an established treatment for end stage cardiac failure.⁴ However transplantation results in denervation of the heart, which persists indefinitely in humans.⁵ Heart transplant recipients have a reduced exercise capacity and an abnormal cardiovascular response to exercise.⁶ ⁷ The chronotropic response of the transplanted heart appears to be heavily dependent on circulating catecholamines.⁸ ⁹ Although transplantation has been found to ameliorate the excessive sympathetic response which is seen during exercise in cardiac failure,⁶ the effect of transplantation, and the consequent cardiac denervation, on sympathetic activity has not been completely defined. The objective of the current study was to investigate the role of the sympathetic nervous system in the exercise response of cardiac transplant recipients. The cardiorespiratory and sympathoadrenal responses of "healthy" transplant recipients, with good left ventricular function, were compared to those of matched normal subjects during progressive dynamic exercise.

Key words: exercise test; transplantation; catecholamines; heart rate; blood pressure.

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Methods

SUBJECTS
Seven male orthotopic cardiac transplant recipients were studied and compared with seven normal male subjects. The cardiac transplant recipients were studied at a mean of 6 (range 3-11) months after transplantation. The indications for transplantation were ischaemic heart disease in three, idiopathic dilated cardiomyopathy in three and congenital heart disease with poor left ventricular function in one. The patients were well, with no clinical signs of cardiac failure. There was no histological evidence of cardiac rejection in their most recent cardiac biopsies (obtained within 4 weeks of the study). Left ventricular systolic function was assessed within 1 month of the study using radionuclide angiography at rest and during steady state supine exercise at 45 watts. The left ventricular ejection fraction was 71 (SEM 3)% at rest and 77(2)% during exercise. Their drug therapy included cardiac denervation was confirmed by absence of heart rate response to carotid sinus massage, slow deep respiration and change in posture.

The normal subjects were selected from the hospital staff to match the transplant recipients, as closely as possible, for age and sex. They were well and had no history of cardiovascular, pulmonary or other significant disease. None was receiving any drug therapy. The mean age of the transplant group was 35.4 (SD 15.3) years and of the controls 35.1(12.7) years (NS); their mean heights were 173(5) cm and 177(6) cm respectively (NS) and their weights 66(7) kg and 73(13) kg respectively (NS). The mean forced expiratory volume in 1 s of the transplant group was 3.6(0.7) litres and of the normals 3.8(0.4) litres (NS); the forced vital capacities of the two groups were 4.4(0.4) litres and 4.8(0.6) litres respectively (NS).

The study protocol was approved by the District Ethics Committee and informed consent was obtained from all subjects.

EXERCISE STUDIES
The subjects were studied in a preprandial state, having fasted for approximately 4 h. The transplant recipients had taken their normal medication in the morning of the study day. An indwelling intravenous cannula was inserted into a forearm vein for subsequent blood sampling and kept patent by intermittent flushing with heparinised saline. After an interval of 10 min the subject was seated on an electronically braked cycle ergometer (Corval Lode, Groningen, Netherlands). Heart rate and ECG recordings were obtained using a computer assisted cardiograph (Marquette MAC II, Marquette, Manchester). Heart rate and a three lead rhythm strip were recorded at 1 min intervals and 12 lead ECGs every 3 min. Blood pressure was measured every 3 min during exercise using a standard mercury sphygmomanometer with the subject’s arm supported at chest level. The subjects breathed room air via a three way valve box (Hans Rudolph, Kansas City, USA). The expired gas was analysed using a Spectramed 9000 computer driven gas analysis system (Cardiokinetics, Salford) which provided minute averaged results for ventilation and oxygen uptake.

Upright exercise was performed on a cycle ergometer. The protocol consisted of 4 min seated at rest, to allow heart rate and ventilation to stabilise, followed by a warm up period of 3 min, cycling at 60 revolutions per minute against a workload of 10 watts. The workload was then increased in 10 watt increments at 1 min intervals to symptom limited maximum exercise. Only the result of the last minute at rest and the last minute of the warm up phase were included in the analysis of the exercise data.

Ten millilitre blood samples were obtained from the indwelling venous cannula just before the start of exercise, at the end of the warm up period, and at alternate minutes during exercise (workload 30 watts, 50 watts etc). The samples were taken into chilled tubes containing lithium heparin and immediately separated by centrifugation at 370 g for 10 min at 4°C. The separated plasma was frozen in liquid nitrogen for subsequent analysis.

Once the subject reached symptom limited maximum exercise, the external workload was removed from the cycle ergometer. The subject was instructed to continue cycling at 60 rpm for 1 min and then stop. During the recovery period heart rate was measured at 1 min intervals and blood samples were obtained at 1, 3 and 6 min after exercise. In one control subject adequate samples could not be obtained in the recovery period.

CATECHOLAMINE ASSAY
Noradrenaline and adrenaline concentrations were measured using high performance liquid chromatography with electrochemical detection.

Extraction — A modified form of the method described by Anton and Sayre was used. Fifty micromol (2.27 pmol) of 3,4-dihydroxybenzylamine was added as an internal standard to 2 ml of plasma. This was followed by 1 ml of tris (hydroxymethyl) methylamine 1 mol-litre\(^{-1}\) (Analar grade from BDH Chemicals, Poole) and ethyldiaminetetraacetic acid 0.05 mol-litre\(^{-1}\) (Sigma Chemicals, Poole) buffered to pH 8.6 with hydrochloric acid. Fifty milligrams of acid washed alumina (Sigma) were then added. The
solution was vortexed for 2 min and then left to equilibrate for 10 min. The supernatant was aspirated and the remaining alumina washed once with 1 ml of tris buffer and thrice with 4 ml distilled water. The catecholamines were back extracted from the alumina with 200 μl of acetic acid 0.2 mol-litre⁻¹ (Aristar grade, BDH). One hundred microlitres of the extract was injected onto the chromatography column. Standard catecholamines for calibration were obtained from Sigma. The catecholamine recovery achieved by the extraction procedure was between 50 and 60%. Extraction losses were accounted for by the use of the internal standard.

CHROMATOGRAPHY — The chromatograph consisted of a Beckman 114M solvent delivery system and a 25 cm, 5 μm, Ultrasphere ODS analytical column (Beckman-RIIIC, High Wycombe) and a Coulochem Model 5100A dual cell electrochemical detector (ESA, Bedford, Mass, USA). The potential of the conditioning cell (Model 5021) electrode was set at +0.10 V and the analytical cell (Model 5011) electrodes set at +0.10 V and -0.38 V. Samples, at a temperature of 4°C, were injected from a CMA 200/240 autosampler (Carnegie Medicin, Stockholm, Sweden). The optimised mobile phase consisted of citric acid 0.03 mol-litre⁻¹ and di-potassium hydrogen orthophosphate buffer 0.015 mol-litre⁻¹ (Anal and Aristar grades respectively, BDH) adjusted to pH 4.7, 1-octanesulphonic acid 2.5 mmol-litre⁻¹ (Aldrich Chemicals, Poole) and 14% methanol (HPLC grade, Rathburn Chemicals, Walkerburn). The mobile phase was recycled at 1.2 ml·min⁻¹. The run time was 20 min, with noradrenaline, adrenaline, and the internal standard having retention times of 9.29, 12.18 and 19.08 min respectively. The data were collected and analysed using a Turbochrom chromatography integration system (Nelson Analytical, Warrington UK). The detection limit for both catecholamines in plasma was 0.02 nmol-litre⁻¹.

Eight normal subjects, studied in our laboratory while resting in the supine position, had noradrenaline concentrations of 2.3 (SD 1.1) nmol-litre⁻¹ and adrenaline concentrations of 0.25(0.11) nmol-litre⁻¹.

STATISTICAL ANALYSIS
The results are given as means (SEM). The baseline characteristics of the two groups and values obtained at maximum exercise were compared by the Mann-Whitney U test. The level of significance was taken as p<0.05. The evolution of the catecholamine and cardiopulmonary response to exercise in the two groups was compared using analysis of variance for repeated measures. The patterns of the response in the two groups were compared by examining the interaction of group and workload or time in the analysis of variance.

Results

BEFORE EXERCISE
All subjects were in sinus rhythm. The control subjects’ ECGs were normal. Three of the transplant recipients had normal ECGs; three had partial and one had complete right bundle branch block. Heart rates were higher in the transplant group at 111(6) v 78(3) beats·min⁻¹ (p<0.01) (fig 1). Systolic blood pressures were higher in the transplant group at 144(5) v 121(3) mm Hg (p<0.01). Diastolic blood pressures [93(2) v 83(5) mm Hg] and noradrenaline concentrations [3.90(0.6) v 2.90(0.2) nmol-litre⁻¹] were also higher in the transplant group but the differences were not statistically significant. There were no differences in ventilation [transplant 9.4(0.8) v control 8.8(0.7) litre·min⁻¹ BTPS] or adrenaline concentrations [transplant 0.26(0.05) v control 0.36(0.06) nmol-litre⁻¹].

DURING EXERCISE
The response at workloads completed by all subjects (up to 80 watts) was compared by analysis of variance. Heart rate, ventilation, noradrenaline concentration, adrenaline concentration, systolic and diastolic blood pressures all increased significantly with exercise (fig 1). There was a significant difference in the pattern of the heart rate response in the two groups, with heart rate accelerating more slowly in the transplant group (p<0.001). Both systolic and diastolic blood pressures were significantly higher in the transplant group (p<0.01) but the pattern of response was similar in the two groups. Ventilation was similar in the two groups up to the 50 watt level but then increased more rapidly in the transplant group (p = 0.05). Noradrenaline concentrations were significantly higher in the transplant group (p<0.05) and there was a trend for the increase during exercise to be greater in the transplant group (p = 0.06). Adrenaline concentrations were an order of magnitude lower than the noradrenaline levels in both groups. There was no difference between the adrenaline levels in the two groups.

PEAK EXERCISE
Each subject exercised to his symptom limited maximum. No adverse reactions or arrhythmias occurred. The symptoms limiting exercise in the transplant group were leg muscle fatigue (4) and breathlessness together with fatigue (3). The symptoms reported by the normal subjects were fatigue (5), breathlessness (1) and both (1).

The maximum workload achieved by the transplant recipients was lower than that of the normal subjects at 102(8) v 170(10) watts (p<0.01), and this was reflected by a lower peak oxygen uptake in the
Before exercise Workload (watts) Workload (watts)

Evolution of the exercise response. Heart transplant group (n=7) ●, normal subjects (n=7) ○. See text for statistical analysis.

transplant group (fig 2). The peak heart rates were significantly lower in the transplant recipients at 142(3) v 181(5) (p<0.01), as was the maximum ventilation at 46(5) v 66(6) litre·min⁻¹ BTPS (p<0.05). There were no significant differences in the peak blood pressures. Because of the lower exercise capacity of the transplant group peak noradrenaline concentrations were similar to those of the normal controls and peak adrenaline concentrations tended to be lower in the transplant group (NS).

RECOVERY AFTER EXERCISE

Heart rate and noradrenaline and adrenaline concentrations all fell significantly during the first 6 min after exercise. Heart rate fell much more rapidly in the normal subjects (p<0.001) and was actually lower than that in the transplant recipients after 2 min (fig 3). There was no significant difference in noradrenaline concentrations between the two groups during recovery. Adrenaline concentrations tended to be higher in the normal subjects (p = 0.07) and fell more rapidly during the recovery period in this group (p<0.05). Catecholamine concentrations in both groups remained higher than the pre-exercise levels during the recovery period.
**FIG 2** Maximum exercise response. Heart transplant group (n=7) ○, normal subjects (n=7) ●; p values refer to Mann-Whitney U test. Individual data points are the mean and SEM for each group are shown.
FIG 3 Pattern of recovery of heart rate (n=7 each group) and catecholamine concentrations (n=7 transplant group, n=6 control group) in the immediate post exercise period. Heart transplant group ● normal subjects ○. See text for statistical analysis.

Discussion

The sympathetic nervous system plays an important role in the cardiovascular response to exercise in normal subjects. The main source of plasma noradrenaline is spillover of noradrenaline released from the axon terminals of sympathetic postganglionic neurones. Circulating noradrenaline concentrations are generally regarded as an index of the overall level of sympathetic activity; however, the sympathetic system is usually activated selectively and regional differences in sympathetic activity often occur. Plasma noradrenaline kinetics are complex. The rate of noradrenaline spillover into plasma depends on sympathetic activity and regional blood flow. Catecholamines are cleared from the extracellular fluid by uptake into postganglionic sympathetic neurones (uptake1) or into extraneuronal cells (uptake2). Theoretically, the increased levels in the transplant recipients might reflect increased spillover into the circulation or decreased clearance. The transplanted, denervated, heart will not contribute to noradrenaline release or uptake by sympathetic neurones. As the innervated heart contributes only 3% of total body noradrenaline spillover and it extracts and releases similar amounts of noradrenaline, denervation would not be expected to affect noradrenaline levels significantly. The normal fall in noradrenaline concentrations during the post exercise period in this study supports the view that clearance is normal in the transplant recipients. Therefore the higher level of noradrenaline in the transplant group appears to reflect increased sympathetic nervous system activity.

The increased sympathetic activity in the transplant group may be a result of their altered cardiac performance and their reduced exercise capacity. Cardiac failure is associated with an increased noradrenaline response during exercise. Cardiac transplantation for end stage cardiac failure has been shown to reduce the sympathetic response during exercise. None of the transplant patients in this study had evidence of cardiac rejection in their most recent cardiac biopsy and their left ventricular function was good. Therefore intrinsic left ventricular dysfunction was not the cause of the increased sympathetic response in these patients.

Efferent cardiac denervation is known to alter the response to dynamic exercise. It results in a sluggish heart rate response to exercise which may affect cardiac output and could alter the sympathetic response to exercise. Transplant recipients are more dependent on changes in stroke volume due to enhanced venous return to increase cardiac output during exercise. Very little increase in cardiac output, however, occurs during the first minute of light exercise. In one study there was an increased arteriovenous oxygen difference in cardiac transplant recipients during exercise, suggesting that cardiac output was reduced in relation to workload and oxygen uptake. In another study, however, the cardiac output of transplant recipients, measured under steady state conditions during submaximal exercise, appeared normal. A reduced, or delayed, cardiac output response during progressive dynamic exercise might produce an increase in sympathetic activity by activation of the arterial baroreceptors. This hypothesis is supported by the observation that...
patients undergoing fixed rate ventricular pacing for complete heart block have a lower cardiac output during exercise than when undergoing atrial synchronous pacing and that this reduced cardiac output is associated with an increased circulating noradrenaline concentration. Hypertension is common in heart transplant recipients treated with cyclosporin and the initial blood pressures of the transplant group were higher than the controls. Blood pressure increased in both groups during exercise. Arterial baroreceptor function is abnormal in cardiac failure but returns to normal as early as two weeks after cardiac transplantation. During exercise, in normal subjects, the arterial baroreceptor reflexes continue to function but are quickly adjusted to a higher "set point", allowing blood pressure to rise. Activation of these reflexes, due to altered cardiac performance, is a possible mechanism for the increased sympathetic activity during exercise.

Loss of afferent input from intracardiac receptors may also increase sympathetic activity during exercise. Ventricular receptors are believed to respond to changes in stroke volume during exercise. In animal experiments tonic input from cardiac receptors has been shown to inhibit sympathetic activity. Loss of this tonic inhibitory input following afferent cardiac denervation could result in an exaggeration of the normal increase in sympathetic activity which occurs during exercise.

Extra cardiac factors may also affect the exercise response and the level of sympathetic activity of the transplant recipients. Deconditioning during the pretransplant illness and the effects of cardiac failure on skeletal muscle may not be reversed immediately after transplantation. The vasodilator response of skeletal muscle to ischaemic stimulus does not return to normal for several months following transplantation. The exercise capacity of heart transplant recipients can be increased by exercise training which may be partly due to improved skeletal muscle performance. It is not known whether the response of blood vessels to sympathetic stimulation is normal in cardiac transplant recipients. In one study higher doses of the α1 agonist methoxamine were required to produce the same increments in blood pressure in heart transplant recipients as in normal subjects. This raises the possibility that there may be a reduced vasoconstrictor response to sympathetic stimulation in transplant recipients and that greater sympathetic activity is required to produce the blood pressure response during exercise.

The increased noradrenaline concentration may help to compensate for the effects of denervation on cardiac performance. β Blockade greatly attenuates the heart rate response to exercise in transplant recipient indicating that circulating catecholamines contribute to the response of the denervated heart. A previous study found a correlation between circulating noradrenaline levels and the rate and contractility of the transplanted heart. In the present study, despite the normal peak noradrenaline concentrations, the transplant recipients achieved lower peak heart rates than the normal controls.

Noradrenaline is not generally considered to function as a circulating hormone, but the levels of circulating noradrenaline reached during heavy exercise, in normal subjects, do have measurable haemodynamic effects. The transplanted heart has an increased sensitivity to circulating β adrenergic agonists. There is evidence for up regulation of β adrenergic receptors in animals following cardiac denervation. The transplanted human heart has an increased sensitivity to infused isoprenaline. The sensitivity to endogenous catecholamines, such as noradrenaline, may be even greater because of loss of uptake by the postganglionic sympathetic neurones in the denervated heart. This does not influence the effect of isoprenaline because it is not a substrate for the uptake process.

The noradrenaline concentrations measured were those in venous blood from a non-exercising limb. At rest, approximately half of the noradrenaline in the venous blood of the forearm originates from the forearm tissues (almost as much noradrenaline has been extracted from the arterial blood by these tissues so that the arteriovenous increment in noradrenaline is small). The levels in arterial blood perfusing the heart may differ by virtue of extraction and release of noradrenaline in the lungs. At rest arterial levels are about 80% of those in the right atrium and about two thirds of those in a brachial vein. Despite these differences and regional variation in sympathetic activity, it seems reasonable to conclude that the increased venous noradrenaline level seen in the transplant group in the present study was associated with an increase in the arterial noradrenaline level.

The noradrenaline and adrenaline response of the normal subjects was similar to that seen in other studies with adrenaline increasing only at the heavier workloads. The adrenaline concentrations were similar in the transplant and control groups. They were a factor of 10 lower than those of noradrenaline in both groups. The levels of adrenaline observed do, however, have a measurable effect on heart rate in normal subjects. Adrenaline and noradrenaline are equipotent at β1 receptors (the predominant receptors modulating chronotropic and inotropic responses in the heart) suggesting that both circulating noradrenaline and adrenaline have an important influence on the cardiac response to exercise in the heart transplant recipients.

During the recovery period the noradrenaline
They had increased circulating levels of noradrenaline remained above pre-exercise levels. The higher heart performance following efferent cardiac denervation, during exercise reflecting increased sympathetic capacity adrenaline act as hormones contributing to cardiac recipients. It appears that both noradrenaline and adrenaline as hormones contributing to cardiac response during exercise in these patients.

References

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