Changes in sarcolemmal Ca entry and sarcoplasmic reticulum Ca content in ventricular myocytes from patients with end-stage heart failure following myocardial recovery after combined pharmacological and ventricular assist device therapy

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Aims Support with left ventricular assist devices (LVAD) improves cardiac performance in patients with end-stage heart failure. In some cases this strategy, combined with pharmacological treatment, has led to a clinical improvement which remained after LVAD explant. This study defines changes in Ca handling at the cellular level in failing left ventricular tissue taken at LVAD implant (LVAD core) and LVAD removal (post-LVAD).

Methods and results We studied cell size and Ca regulation in enzymatically dissociated cardiac myocytes. We used confocal microscopy and electrophysiological techniques to investigate the SR Ca content and major Ca movements across the sarcolemma during the action potential. We firstly recorded a significant reduction in cell capacitance and cell volume consistent with regression of cellular hypertrophy in post-LVAD myocytes compared with LVAD core myocytes. Ca entry via sarcolemmal Ca channels during the action potential using action potential voltage-clamping was significantly increased in post-LVAD myocytes compared with LVAD core myocytes. Finally, SR Ca content (assessed by integrating the caffeine-induced Na/Ca exchanger transient inward current) in post-LVAD myocytes was also significantly increased compared with LVAD cores myocytes.

Conclusions These results show that in myocytes from patients after LVAD support there is more Ca entry to trigger Ca release and more SR Ca content, leading to improved contractile function.

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KEYWORDS
Heart-assist device; Heart failure; Calcium; Sarcoplasmic reticulum; Myocytes

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Introduction

Long-term left ventricular assist device (LVAD) support improves survival and quality of life in patients with advanced heart failure and contraindications to cardiac transplantation. LVAD support is also routinely used as a bridge to transplantation in patients with end-stage heart failure. In a number of patients a significant improvement of myocardial performance has been achieved and the device has been explanted. Preliminary observations using LVAD implantation (Thermocardiosystem vented electric Heart Mate 1) associated with combination pharmacological therapy (β-blockers, ACE-inhibitors, angiotensin II receptor antagonists and spironolactone, followed by administration of the β2 receptor agonist clenbuterol; ‘Harefield regimen’) suggest a role for this strategy as a ‘bridge to recovery’ in the treatment of end-stage heart failure. The molecular and cellular mechanisms, frequency and durability of ‘recovery’ remains largely unknown. Many hypotheses have been put forward, considering the mechanisms underlying heart failure particularly regarding the role of Ca. Well known changes in protein expression for the sarcoplasmic reticulum Ca ATPase (SERCA), Na/Ca exchanger and phospholamban seem to be key elements of myocyte dysfunction and have been proposed as possible target for treatment. Gene therapy to increase expression of SERCA has been proposed and found efficacious in cardiac myocytes. The role of the overexpression of the Na/Ca exchanger found in some studies is controversial, and discussion is ongoing on whether this mechanism acts as compensation for or a cause of heart failure. Some studies have compared contraction characteristics of myocytes from non LVAD-treated explanted hearts with myocytes isolated from LVAD-treated hearts and shown a beneficial effect of mechanical support on the velocity of contraction. Modifications of the Ca current properties were found in another study. The causes of these changes need further investigation. In the present study we investigated Ca regulation mechanisms and contraction patterns of isolated cardiac cells from tissue obtained from patients during LVAD implantation and after clinical improvement following treatment with the Harefield Regimen. We compared results with data obtained from donor heart tissue. We found that the combined pharmacological and mechanical therapy increased the velocity of contraction and relaxation, increased L-type Ca current and increased SR Ca load. These findings were not accompanied by normalisation of the force–frequency relationship supporting the hypothesis that functional compensation rather than normalisation effects may be involved in the myocardial recovery obtained with the combination therapy.

Methods

Patients

Tissue was obtained from seven patients in class IV NYHA, with normal coronary arteries, during implantation of the LVAD (LVAD core). Using myocardial biopsies, tissue was also taken from six patients during LVAD explant or from explanted hearts when the patients with LVAD support underwent cardiac transplantation (post LVAD). During LVAD support the patients were treated with a combination therapy (‘Harefield Regimen’) composed of mechanical support and administration of beta-blockers, ACE-inhibitors, angiotensin II receptor antagonists and spironolactone. This was followed by the administration of the β2 receptor agonist clenbuterol, which stimulates physiological hypertrophy and improves cardiac function. All the patients showed significant myocardial improvement off-pump compared with the time of implantation. Biopsies obtained from four donor hearts were used as control. The study was given ethical approval by the Brompton, Harefield and NHLI Ethics Committee. Informed consent was obtained from all the patients.

Cell isolation

Ventricular tissue was placed in ice-cold cardioplegia and transported to the laboratory where cardiac myocytes were enzymatically isolated using a technique previously described. Briefly, tissue was finely chopped using razor blades. It was then washed and oxygenated in a nominally zero-Ca solution containing (in mM) NaCl, 120; KCl, 5.4; MgSO4, 5; pyruvate, 5; glucose, 20; taurine, 20; HEPES, 10; nitrolotriacetic acid (NTA), 5 (bubbled with 100% O2; pH=6.8) for 12 min, changing the solution every 3 min. The tissue was then gently shaken in enzyme solution (in mM: NaCl, 120; KCl, 5.4; MgSO4, 5; pyruvate, 5; glucose, 20; taurine, 20; HEPES, 10; nitrolotriacetic acid (NTA), 5 (bubbled with 100% O2; pH=6.8) for 12 min, changing the solution every 3 min. The tissue was then gently shaken in enzyme solution (in mM: NaCl, 120; KCl, 5.4; MgSO4, 5; CaCl2, 0.2; pyruvate, 5; glucose, 20; taurine, 20; HEPES, 10; bubbled with 100% O2; pH=7.4) containing protease (4 U/ml, Sigma) for 45 min and collagenase (1 mg/ml, Sigma) for another 45 min. A further 45 min digestion in collagenase followed. At the end of every cycle, the suspension was gently spun (500 cpm) and the pellet re-suspended in enzyme solution. Cells were
kept at room temperature. Rod-shaped, Ca tolerant myocytes were used within 5–6 h from isolation.

**Cell size determination**

Two techniques were used to assess the size of isolated myocytes: confocal microscopy and electrophysiology. Ventricular myocytes were suspended in normal Tyrode (NT) solution containing 5% glutaraldehyde. After 24 h, the supernatant was discarded and the cells re-suspended in fresh NT solution. The experimental chamber was mounted on a Nikon TE300 with Bio-Rad Radiance 2000 confocal attachment and myocytes observed through a Nikon Plan-Fluor ×40 oil-immersion lens (N.A.=1.3) and the confocal pinhole set to 1.2 µm to obtain the best spatial resolution. 488 nm excitation line of an argon laser was used. Emitted fluorescence from glutaraldehyde (no need for the use of other fluorescent dyes) was collected through a 500 nm long-pass filter. Confocal image series at 1.5 µm on the z-axis were acquired. Cell volume was calculated by 3-D rendering of these confocal images (Laservox 1.0 Bio-Rad). The spherical aberration inherent to our objective was calculated by using fluorescent spheres (FluoSpheres, Molecular Probes Europe BV, The Netherlands). A correction factor of 0.74 was found to abolish the distortion of the images and therefore subsequently used in the quantification of the cell volumes.

Myocyte surface area was calculated as a function of cell capacitance during the electrophysiological experiments in freshly isolated myocytes. For this purpose we used the Membrane Test protocol performed by pClamp7 (Axon). This protocol was employed with pulses from −80 to −85 mV at 100 Hz frequency. Cell capacitance (Cm) was calculated from the formula:

$$C_m = (Q_1 + \Delta I \tau) / \Delta V$$

where Q1 is the charge in the transient above the steady-state response; $\Delta I$ is the difference of the current before and at the end of the pulse, $\tau$ is the time constant of decay and $\Delta V$ is the difference in voltage.

We also calculated the volume: capacitance ratio. This index is well known in other species but has never been measured in human cells. Since the measurements of volume and capacitance were performed on different cells from the same preparation, we averaged the values for these parameters from each patient and then ratioed the values. The n number for the volume: capacitance ratio therefore represents the number of patients.

**Functional assessment**

**Contraction experiments**

Myocytes were placed in a chamber on the stage of an inverted microscope and superfused with Krebs-Henseleit (K-H) solution (mM): NaCl, 119; CaCl2, 1; KCl, 4.7; MgSO4, 0.94; KH2PO4, 1.2; NaHCO3, 2; and glucose, 11.5) that was bubbled with 95% O2–5%CO2 to pH 7.4. Experiments were carried out at 37 °C with field stimulation at 0.2 Hz and contraction was monitored by a video edge detection device with a spatial resolution of 1 in 256 or 512 and a time resolution of 10 or 20 ms.

Myocytes were randomly selected for 5-min recordings of contraction amplitude (percentage cell shortening), time-to-peak contraction (TTP) and time-to 50% (R50) and 90% (R90) relaxation. Determination of contractile response to increasing frequency was also performed at this Ca concentration.

**Electrophysiology**

The cells were superfused with Normal Tyrode solution (mM): NaCl, 140; KCl, 6; MgCl2, 1; CaCl2, 1; glucose, 10; HEPES, 10; pH to 7.4 with 2 M NaOH). The electrophysiological experiments were performed using an Axoclamp-2B system (Axon Instruments). To avoid dialysis of the cells and to minimize the effects of changing the intracellular environment, high resistance microelectrodes (15–25 MΩ; Clark Electromedical Instruments) were used. The microelectrode filling solution contained: KCl, 2 M; EGTA; 0.1 mM; HEPES, 5 mM, pH=7.2. The switching rate was 5–7 kHz. Gain was increased up to 0.8 nA/mV to obtain a maximally square voltage trace with no oscillations. Current clamp and voltage clamp protocols were controlled with pClamp 7 software (Axon Instruments). Action potentials were recorded in current clamp mode after stimulating the cells with a 1 nA current pulse (5 ms duration). Measurement of SR Ca content was made using a method described in detail elsewhere. Rapid application of caffeine followed a stimulation train of voltage clamp steps from −80 to 0 mV (1 Hz). Caffeine was added in solid form to Normal Tyrode solution to give a final concentration of 20 mM. To measure the current-voltage relationship of the L-type calcium current cells were depolarised with a series of 500 ms pulses from a holding potential of −40 mV to potentials between −50 and +40 mV each preceded by stimulation trains of voltage clamp pulses from −80 mV to 0 mV for 500 ms (1 Hz). A similar series of pulses were then imposed on the cell in the presence of 200 µM Cd.
Solutions

Chemicals were purchased from BDH (Poole, UK). Caffeine (20 mM) was added as solid to the final solution. The temperature of the superfusing solution was approximately 37 °C. The rate of superfusion was 2–3 ml min−1 except during fast application of caffeine when it was 12–15 ml min−1. Miniature solenoid valves (The Lee Company, Essex, UK) were used to produce fast changes in the superfusate. These changes were electronically controlled using pClamp7 (Axon Instruments) trigger outputs.

Data acquisition and statistical analysis

The data obtained from the Axoclamp-2B system was recorded on a computer using pClamp7. The rate of sampling was between 0.5 and 3 kHz. To assess statistical differences between means, a one-way ANOVA with Bonferroni post test was used: p<0.05 was considered significant. Unless otherwise specified, the results are expressed as mean±standard error of the mean (SEM). The n number represents the number of patients that have undergone investigation. Each value is calculated as the average of the values recorded from single cells isolated from a single preparation. A minimum n number of 4 was used.

Results

Cell size determination

Using confocal microscopy we performed a 3-D reconstruction of cell volume. We found that cells from LVAD cores were approximately doubled in volume compared with donor cells. The volume of post LVAD cells returned to values comparable with donor cells. Data are shown in Fig. 1.

Cell capacitance is an index of cell surface area and can be calculated during electrophysiological
experiments. Cell capacitance was increased approximately three-fold in cells from LVAD candidates compared with donor cells as shown in Fig. 1. Cell capacitance was reduced in post-LVAD myocytes. The ratio between cell capacitance and volume (calculated from the same patient) was significantly increased in cells from LVAD cores suggesting that there was more surface per volume unit in these cells. This result may suggest changes in the T-tubular structure as previously detected (e.g.21). No significant difference was found between LVAD core and post-LVAD myocytes.

Contraction patterns

Myocytes from LVAD cores, field-stimulated at 1 Hz, had a slower time-course of contraction and relaxation compared with the post-LVAD group. These results are shown in Fig. 2. Speed of contraction and relaxation in post LVAD myocytes was not different from these parameters measured in donor heart myocytes. Cell contraction is regulated predominantly by changes in intracellular [Ca]. Several mechanisms, including sarcolemmal Ca channels, SR uptake and release, Na/Ca exchanger and other minor mechanisms are involved in determining the

Fig. 2 Changes in patterns of contraction in isolated myocytes following LVAD support. Ventricular myocytes were field stimulated and cell shortening was measured (as percentage of resting cell length) using a video edge detection system. Panel A shows original traces from an LVAD core myocyte and from a post LVAD myocyte at 0.2 and 1 Hz (left, upper section). The bar graphs describe mean data for time-to peak of contraction (TTP) and time to 50% (R50) and 90% (R90); n>5. Panel B describes the shortening–frequency relationship in the three groups. *p<0.05; **p<0.01.
properties of the Ca transients and therefore contraction and relaxation. Because of the high feedback that regulates these mechanisms, one cannot infer, from contraction experiments alone, if the return towards normal values in post LVAD tissue was due to normalisation of the mechanisms involved or to compensatory action mediated by other mechanisms. The following experiments investigate the specific changes in the function of Ca regulatory proteins in LVAD cores and the effects of the combination therapy on these measurements.

**Shortening–frequency relationship**

Fig. 2 shows the shortening–frequency relationship in the three groups of myocytes studied. Myocytes from donor hearts showed a positive shortening–frequency relationship, whereas in LVAD core myocytes the frequency-dependent increase in contraction was not observed. Similar behaviour was previously reported in myocytes from failing hearts. Interestingly, a normalisation of the shortening–frequency response was not observed in post-LVAD myocytes, suggesting that clinical improvement associated with faster contraction and relaxation was not accompanied by a positive shortening–frequency relationship. Similar findings were previously reported when post LVAD tissue from explanted hearts was compared to tissue obtained from explanted hearts without LVAD.

**Sarcolemmal Ca current**

An essential regulatory property of cardiac muscle is a graded response that depends on the size of the Ca trigger. The predominant source of trigger for Ca release is the Ca current flowing through the voltage-activated Ca channels. In order to investigate the properties of L-type Ca channels, cells were voltage-clamped at −40 mV and depolarised to variable voltages up to +40 mV for 400 ms. The protocol was repeated in the presence of 200 µM Cd. A typical bell-shaped current–voltage relationship was achieved (Fig. 3). In LVAD core myocytes there was a significantly reduced amplitude of the Cd-sensitive current at most voltages compared with donor myocytes and post-LVAD myocytes (there was no difference between these two groups). Although other factors, such as the amount of Ca release from the SR could be important, in LVAD candidates sarcolemmal Ca channels have reduced current amplitude. After LVAD implant these parameters return to value similar to donor cells.

A potential limitation of these experiments is that, although they assess properties of the Ca channels in an intact environment, they do not consider variations in the action potential morphology, which is widely documented in heart failure. In order to address this point we performed experiments using a technique previously validated in guinea-pig and rat myocytes, which measures the amount of Ca entry via the sarcolemmal Ca channels during the action potential recorded from the specific cell, hence considering variations in action potential morphology. Briefly, action potentials were recorded at 1 Hz and in the same cells used for action-potential voltage clamping (e.g. the cell action potential was the waveform during voltage-clamp experiments). Cd (2 mM) was rapidly applied to inhibit both Ca channels and Na/Ca exchanger. The resulting Cd-sensitive current was the summation of both inward currents produced by Ca entry via the sarcolemmal Ca channels and Ca extrusion via the electrogenic Na/Ca exchanger. Assuming that Ca channels remain the predominant route for Ca entry and the Na/Ca exchanger the main mechanism of Ca extrusion from the cell (so that at the steady-state the two are balanced), one can derive Ca flowing through Ca channels from the size of this Cd-sensitive current (which should describe the amount of Ca moving across the cell membrane at every beat). As shown in Fig. 4, in LVAD core myocytes, this current was again reduced compared with both donor and post-LVAD myocytes suggesting that the contribution of sarcolemmal Ca movement to excitation–contraction...
coupling in failing myocytes is reduced and there is a recovery of such a mechanism after the LVAD implant. Because of the different changes in cell volume and cell capacitance in our groups (Fig. 1), we first chose to normalise the Ca current to cell capacitance (pC/pF) because this value better describes the Ca flux per unit of sarcolemma (Fig. 4). This is more relevant to describe the function of the Ca current as a trigger for SR Ca release and its role in the local control of excitation–contraction coupling. Normalising the Cd-sensitive current to cell volume better describes the contribution of the sarcolemmal Ca movements to bulk cytoplasmic [Ca]. When the integral of the Cd-sensitive current was normalised to cell volume there was no statistically significant difference between the groups (donor: 1.624±0.553 pC/pl (n=4) (mean±SEM); LVAD core: 0.641±0.09 pC/pl (n=5); post LVAD: 2.209±0.52 pC/pl (n=5)). This may suggest that the contribution of the Ca current to bulk cytoplasmic [Ca] is less affected by heart failure or may reflect a low power of the statistical test due to the small number of samples. More experiments are required to specifically address this point.

**SR Ca content**

We performed experiments to assess the ability of the SR to store and release Ca before and after LVAD implant (Fig. 5). We used an established technique based on measuring the caffeine-induced transient inward current ascribed to the Na/Ca exchanger allows quantification of the amount of releasable Ca from the SR. Since we found a different surface:volume ratio in the different groups, quantification of SR Ca content was performed using the appropriate factors to convert cell capacitance in cell volume from Fig. 1 (donor: 3.5 pF/pl; LVAD core: 6.44 pF/pl post LVAD: 5.03 pF/pl. We assumed that mitochondria accounts for 33% of the cell volume. In post-LVAD myocytes there was a significant increase in the SR Ca content compared with myocytes from donor hearts and from LVAD cores. *p<0.05. (n: control: 7/4; LVAD core: 11/6; post LVAD: 14/6; number of cells/number of patients).
current allows quantification of SR Ca content. We calculated that charge flowing during this current was \(0.64\pm0.16 \, \text{pC/pF} \) (\(n=4\)) in donor samples, \(0.43\pm0.09 \, \text{pC/pF} \) (\(n=7\)) in LVAD cores and \(1.15\pm0.25 \, \text{pC/pF} \) (\(n=6\)) in post-LVAD samples. For the calculation of \([\text{Ca}]\), the appropriate conversion factor of capacitance to volume as calculated in Fig. 1 was used. We found that SR Ca content was unchanged in LVAD core compared with donor but in post-LVAD tissue this parameter was significantly increased (\(30.9\pm8 \, \mu\text{M/l non-mitochondrial volume} \) (\(n=4\)) in donor samples, \(39\pm8.2 \, \mu\text{M/l} \) (\(n=7\)) in LVAD cores and \(90.6\pm17 \, \mu\text{M/l} \) (\(n=6\)) in post-LVAD samples; \(p<0.01\)). This is an important finding because it suggests that SR Ca is maintained in failing myocardium. We do not know whether in our samples uptake and release mechanisms are affected. Importantly, in the post LVAD tissue there was a significant increase in SR Ca content. This finding associated with a return to normal Ca trigger may explain the faster contraction and relaxation observed.

**Discussion**

In this study we have shown the pattern of changes in Ca entry via the Ca channels during the action potential and in the SR Ca content following clinical recovery utilising LVAD combination therapy.

**Changes in morphology and Ca regulation of myocytes from patients in end-stage heart failure**

Cardiac tissue removed during LVAD implant in patients in end-stage heart failure showed distinct changes in size and in Ca regulation. Cell volume and cell capacitance were both increased in LVAD core myocytes indicating cellular hypertrophy. Critically, the increase in cell capacitance was larger than the increase in cell volume. This resulted in a significantly larger capacitance:volume in failing myocytes compared with myocytes from donor hearts.

This finding may represent an effect of remodelling based on larger growth of the cell membrane in hypertrophy compared with cell volume and may reflect disruption in t-tubular morphology. Changes in t-tubular morphology in failing myocardium have been reported previously, although other studies found no changes in these parameters. This change in surface area to volume ratio is an important finding for the interpretation of electrophysiological data. Normalisation of membrane currents to cell capacitance is common practice and assumes that cell capacitance reflects cell size with a fixed relationship (as showed in normal myocytes from different species). In our experiments when failing myocytes were studied, this relationship varied. This has important consequences for the interpretation of the results. When the SR Ca content per unit of cell volume is calculated from the caffeine-induced inward current, capacitance must be converted into volume using the appropriate value derived from Fig. 1. Using this conversion we detected no difference in SR Ca content between donor and failing (LVAD core) myocytes. If a standard conversion factor (e.g. \(5 \, \text{pF/pl}^{26}\)) was used for both groups, the data would incorrectly show an apparent reduction of SR Ca content in failing myocytes, although this would not be significant. Establishing the actual capacitance to volume ratio is therefore essential for correct estimation of SR Ca content.

Previous studies have shown that SR Ca content is reduced in failing human cardiac myocytes compared with non-failing myocytes and is in contrast with our present findings. Several reasons can explain this discrepancy. For example, the population of patients and the source of the control tissue differ. In our study only tissue from patients with dilated cardiomyopathy and normal coronary arteries undergoing LVAD implantation was used. Control tissue was taken only from used donor hearts. Unused hearts show modifications in Ca handling proteins and their role as control is debatable. Moreover, the tissue was taken exclusively from the apex of the left ventricle. Lindner et al. measured SR Ca content using rapid caffeine applications and Ca-sensitive fluorescent indicators. Fluorescent indicators have technical limitations such as saturation, different protein binding in different conditions, caffeine-induced quenching. In particular the indicators introduce an additional source of Ca buffer that makes quantification unreliable. In this study we have used the integral of the caffeine-induced current alone to avoid this technical problem. Integrating the caffeine-induced current, Piacentino et al. found that SR Ca content from failing hearts was reduced compared with non-failing hearts. However, the same capacitance-to-volume conversion factor was used for both groups (13 pF/pl). The present study is the first attempt in which the conversion factor between cell capacitance and volume was measured in the same population of human ventricular myocytes under investigation and used for the calculation of cell volume.
We are aware of some limitations in our assessment of this conversion factor and more studies are required. Possible sources of error are the calculation of the cell volume and cell capacitance from different cells, which may increase the variability of the data. This was unavoidable in the present study because of the limited resources and amount of tissue available. The usage of fixed cells, which allowed the measurement of cell volume on cells from the same sample later in time, is also not ideal since this could produce a minimal cell shrinkage and cause a slight underestimation of cell volume with consequent artifactual increase in the capacitance:volume ratio. It remains that, since such an effect would equally affect all preparations, this would not alter the qualitative validity of our results. Our observations remain important because they demonstrate that assuming a similar capacitance:volume ratio in different pathological conditions can be misleading and specific quantification is required.

Effects of the combined pharmacological and mechanical therapy on cell dimensions and Ca regulation

The clinical improvement of the patients undergoing the Harefield Regimen was associated with changes at the cellular level. There was a clear reduction in cell size, expressed as reduction in both cell capacitance and cell volume, although the cell capacitance did not decrease to control level so that the ratio capacitance:volume did not reach statistical difference with the LVAD core myocytes. Cell relaxation was faster, and this was associated with normalisation of the Ca current and increased SR Ca content.

One major problem with the use of mechanical unloading in the treatment of heart failure is the consequent heart muscle atrophy. This could be an important impediment to the removal of the LVAD when the reverse remodelling has been achieved, limiting the efficacy of the treatment. Clenbuterol, a β2 agonist known for its anabolic effects on skeletal muscle could be of value in this regard and was administered after LVAD implant. We have previously shown that clenbuterol induces a physiological hypertrophy of the heart without significant necrosis or interstitial fibrosis. In addition we reported that clenbuterol, in conjunction with pressure overload hypertrophy by aortic banding, produces a specific type of cardiac hypertrophy with preserved LV function, less fibrosis and greater expression of SERCA2a mRNA than aortic banding alone. It should be stressed that the efficacy of the treatment with clenbuterol was not investigated in this study because all the patients were treated with this drug and comparison with a non-treated group was not attempted. The specific role of chronic treatment with clenbuterol on excitation–contraction coupling in heart failure and recovery requires further investigation.

The specific pharmacological regime used in this study may be responsible for differences in our data compared with findings from other groups. Chen et al., for example, found that the amplitude of the Ca current was unchanged in the post LVAD group, although they found modifications in the inactivation of this current. Dipla et al. detected a more positive force–frequency relationship in the post LVAD group. Together with possible differences in the aetiology, severity of the disease and the pharmacological regime used in other studies, another possible explanation is the difference in electrophysiological technique employed. We utilised high resistance microelectrodes, which minimise the dialysis of the cells. This is unavoidable with the use of larger patch-clamp electrodes and can influence the behaviour of ion channels and Ca regulatory mechanisms.

Parameters that regress to control and parameters that remain unaltered after LVAD

From our results it appears that the clinical improvement of patients treated with the Harefield Regimen is associated with regression of changes at a cellular level. This includes a faster contraction and relaxation, a normalization of the current–voltage relationships and Ca entry during the action potentials. Another effect that can be associated with functional improvements is an increased SR Ca content in the post-LVAD group. The force–frequency response remained blunted and did not recover after LVAD. The relationship between cell capacitance and volume did not reach values comparable with the donor hearts although it was reduced compared with failing myocytes. We would like to speculate that these changes (morphological variations of t-tubular structure, blunted force-frequency) can be a feature of hearts potentially going into failure and can be present even before the onset of clinical signs. They may represent some of the defects of the completely unexplored asymptomatic or mild failing human heart. These changes may predispose to conditions that precipitate the clinical picture and are associated, as shown in this study, to disruption of Ca trigger.
LVAD implant associated with pharmacological treatment is able to bring the patients back to more stable haemodynamic conditions. Factors that predispose to acute failing events remain. Characterisation of patients that can benefit from the treatment and the natural history of such recovery require further clinical and cellular studies.

In conclusion, this study has shown that in myocytes from patients in end-stage heart failure there is a predominantly increased cell surface area compared with cell volume, a reduced Ca entry via sarcolemmal Ca channels and a blunted shortening–frequency relationship. In myocytes isolated after myocardial recovery using combined pharmacological and mechanical therapy, there is more Ca entry to trigger Ca release and more SR Ca uptake during the cardiac cycle, leading to improved diastolic function. However in these myocytes shortening–frequency stays blunted.

These results provide unequivocal support to the role of changes in Ca homeostasis in the pathophysiology of heart failure. They also provide positive evidence for the role of a combination of pharmacological and mechanical therapy in producing regression of cellular dysfunction in patients in end-stage heart failure.

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