

# Contractile function is preserved in unloaded hearts despite atrophic remodeling

Henriette Brinks, MD,<sup>a</sup> Hendrik Tevaearai, MD, MBA,<sup>a</sup> Christian Mühlfeld, MD,<sup>b</sup> Daniela Bertschi, MD,<sup>a</sup> Brigitta Gahl, MSc,<sup>a</sup> Thierry Carrel, MD,<sup>a</sup> and Marie-Noelle Giraud, PhD<sup>a</sup>

**Objective:** Recent studies have shown that mechanically unloading a failing heart may induce reverse remodeling and functional improvement. However, these benefits may be balanced by an unloading-related remodeling including myocardial atrophy that might lead to decrease in function. Using a model of heterotopic heart transplantation, we aimed to characterize the myocardial changes induced by long-term unloading.

**Material and Methods:** Macroscopic as well as cellular and functional changes were followed in normal hearts unloaded for a 3-month period. Microscopic parameters were evaluated with stereologic methodology. Myocardial contractile function was quantified with a Langendorff isolated, perfused heart technique.

**Results:** Atrophy was macroscopically obvious and accompanied by a 67% reduction of the myocyte volume and a 43% reduction of the interstitial tissue volume, thus accounting for a shift of the myocyte/connective tissue ratio in favor of noncontractile tissue. The absolute number of cardiomyocyte nuclei decreased from  $64.7 \pm 5.1 \times 10^7$  in controls to  $22.6 \pm 3.7 \times 10^7$  (30 days) and  $21.6 \pm 3.1 \times 10^7$  (90 days) after unloading ( $P < .05$ ). The numeric nucleic density in the unloaded myocardium, as well as the mean cardiomyocyte volume per cardiomyocyte nucleus, remained constant throughout the 90 days of observation. Functional data indicated an increase in ventricular stiffness, although contractile function was preserved, as confirmed by unaltered maximal developed pressure and increased contractility (maximum rate of left ventricular pressure development) and relaxation (minimum rate of left ventricular pressure development).

**Conclusion:** Atrophic remodeling involves both the myocyte and interstitial tissue compartment. These data suggest that although there is decreased myocardial volume and increased stiffness, contractile capacity is preserved in the long-term unloaded heart.

Treatment of severe heart failure may involve implantation of a mechanical ventricular assist device (VAD). VAD-mediated ventricular unloading can lead to reversal of hypertrophy and functional improvement, but rarely to lasting recovery and device explantation.<sup>1-4</sup> Intense research into the underlying cellular and molecular mechanisms has identified “reverse remodeling,” a process composed of normalization of myocardial morphology,<sup>5,6</sup> calcium handling,<sup>7</sup> beta-adrenergic response,<sup>8,9</sup> and molecular signaling factors.<sup>10</sup> Furthermore, clinical studies have shown adaptive changes to mechanical unloading, including fibrosis of the myocardium and atrophy of cardiomyocytes,<sup>11,12</sup> two features considered harmful, which consequently might preclude functional recovery. Relative increase of fibrotic tissue,<sup>13</sup> as well as macroscopic and microscopic atrophy of the myocardium, were also found in experimental models

of mechanical unloading involving hypertrophied and ischemic failing rat hearts.<sup>14,15</sup> Inasmuch as these data involved failing hearts, the unloading-related atrophic remodeling including deleterious consequences is difficult to differentiate from beneficial reverse remodeling.

In the current study, we characterize long-term morphologic, cellular, and functional changes occurring in the chronically unloaded normal hearts to investigate consequences of atrophic remodeling without interference of previous myocardial damage.

## METHODS

### Animals

Male Lewis rats (300–350 g) were used as donors and recipients throughout the study. All animals received humane care in compliance with the Swiss Animal Protection Law after permission had been obtained from the State Veterinary Office, Berne, Switzerland.

### Heterotopic Transplantation

Heterotopic heart transplantation was accomplished as previously described.<sup>16,17</sup> In brief, heart transplantation is done by anastomosing end to side the ascending aorta of the donor heart to the abdominal aorta of the recipient. The pulmonary artery of the transplant is anastomosed end to side to the vena cava. Coronary arteries are perfused normally and the heart beats in a regular sinusal way. The left ventricular (LV) and left atrial cavities remain unloaded. The control group consisted of animals that underwent laparotomy and clamping of the abdominal vessels, as well as incision and resuturing of the aorta and vena cava (control animals were humanely killed 30 days after the sham operation).

From the Department of Cardiovascular Surgery, Inselspital, Berne University Hospital and University of Berne,<sup>a</sup> and the Institute of Anatomy, University of Berne,<sup>b</sup> Berne, Switzerland.

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Address for reprints: Hendrik Tevaearai, MD/MBA, Laboratory of Cardiovascular Surgery, Murtenstrasse 35, CH-3010 Berne, Switzerland (E-mail: [hendrik.tevaearai@insel.ch](mailto:hendrik.tevaearai@insel.ch)).

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### Abbreviations and Acronyms

$dP/dt_{max}$	= maximum rate of left ventricular pressure development
$dP/dt_{min}$	= minimum rate of left ventricular pressure development
LV	= left ventricular
VAD	= ventricular assist device

### Macroscopic Analysis

LV weight was measured before implantation and again after the animals were humanely killed at 3, 8, 15, 30, 60, and 90 days ( $n = 15/\text{group}$ ). Corresponding muscle volume was calculated by dividing this weight by the tissue density of rat muscle ( $1.06 \text{ g/cm}^3$ ).<sup>18</sup> Comparison of the obtained LV mass was done for the value at the time of transplantation and after unloading time period (Figure 1, A).

### Stereology

Stereologic analyses were based on two consecutive sections of ventricular tissue, hematoxylin–eosin and trichrome stained, to obtain physical disector pairs with a disector height of  $5 \mu\text{m}$  and analyzed with a microscope equipped with a computer-assisted stereology tool (CAST 2.0; Olympus, Ballerup, Denmark).<sup>19</sup> Quantifications were performed as detailed in supplementary data. Control hearts obtained from animals 30 days after sham operations were used as controls.

### Functional Assessment

Isovolumetric LV pressure measurements were performed as previously described<sup>20,21</sup> with an intraventricular balloon connected to a pressure transducer–tipped catheter (Millar Instruments, Inc, Houston, Tex).

### Statistical Analysis

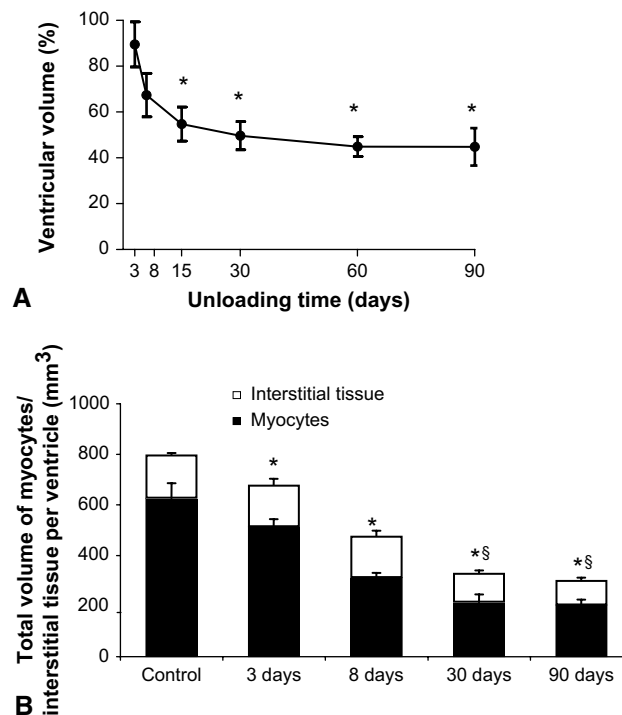
Results are presented as mean  $\pm$  SD. One-way analysis of variance was used to compare groups followed by Bonferroni post hoc tests for LV dimensions and stereologic analysis. A nonparametric Mann–Whitney test was used to compare the slope of the end-diastolic volume–pressure relations. Analysis of variance for repeated measurements was applied to test the volume–pressure and rate of pressure development.

## RESULTS

### Morphologic Changes

Morphologic measurements revealed remodeling of ventricular size (Table 1). At a macroscopic level, reductions in LV mass compared with the LV mass at implantation occurred rapidly within 3 days with stabilization after 30 days (Table 1, Figure 1, A).

At a cellular level, LV wall sections were analyzed by stereologic methods to accurately quantify cellular modifications. Two aspects reflected atrophic remodeling. First, the total volume of myocyte and interstitial compartments rapidly and significantly decreased, reaching a maximum after 30 days (Figure 1, B). Volume of cardiomyocytes decreased to a larger extent than interstitial tissue, resulting in a proportional increase of interstitial tissue compared with the volume of cardiomyocytes. The fraction of myocytes rapidly and significantly decreased from  $78\% \pm 2\%$  ( $n = 5$ ) in con-



**FIGURE 1.** A, LV mass decreases in a time-dependent manner after unloading. Mass was calculated from weight of the ventricles divided by the tissue density of rat muscle ( $1.06 \text{ g/cm}^3$ ).  $*P < .05$ . B, Stereologic volume analyses ( $n = 5$  per groups). Total volume of myocytes in the ventricles significantly decreased after 3 days of unloading ( $*P < .05$  vs control).

trol hearts to  $65\% \pm 3\%$  at 8 days after transplantation, and this proportion remained stable over the 90 days of our observation.

Second, stereologic quantification of cardiomyocyte nuclei demonstrated that the total number of cardiomyocyte nuclei decreased significantly after 8 days compared with control (Table 2). In contrast, the number of cardiomyocyte nuclei per cubic micrometer (ie, the numerical density of nuclei) remained constant throughout the 90 days. Mean cardiomyocyte volume per cardiomyocyte nucleus also remained unchanged. Taken together, these results suggest that the reduction in myocardial wall volume is a result of a reduction in myocyte and interstitial volumes; however, cellular response to unloading is accompanied by an adaptive process that preserves a constant numerical density of cardiomyocyte nuclei and a constant ratio of cytoplasmic volume per nucleus.

### Ventricular Function

**Contractile function.** Isovolumetric LV pressure measurements were performed. Cardiac contractile function was assessed by recording the maximum and minimum rates of LV pressure development (respectively,  $dP/dt_{max}$  and  $dP/dt_{min}$ ). Stepwise increments of volume in the fluid-filled balloon inserted into the LV were used to

TABLE 1. Macroscopic characterizations of the LV atrophy

Parameter	Control	3 days	8 days	30 days	60 days	90 days	P value
Body weight (g)	336 ± 32	360 ± 50*	350 ± 10*	430 ± 23*	450 ± 18*	480 ± 20*	.001
LV weight (g)	0.811 ± 0.071	0.769 ± 0.059*	0.535 ± 0.043*	0.356 ± 0.030*	0.329 ± 0.023*	0.323 ± 0.027*	.0001
Myocardial wall volume (cm <sup>3</sup> )	0.765 ± 0.067	0.725 ± 0.055*	0.505 ± 0.040*	0.336 ± 0.027*	0.310 ± 0.021*	0.305 ± 0.029*	.0001
LV length (mm)		14.5 ± 1.5	12.4 ± 0.8†	9.9 ± 0.7†	9.2 ± 0.5†	9.4 ± 1.0	.0001
LV width (mm)		12.3 ± 0.6	10.8 ± 0.9‡	9.9 ± 0.6†	9.7 ± 0.5†	9.4 ± 0.8	.0001

LV, Left ventricular. P values represent the effect of time of unloading on parameters (1-way analysis of variance). Significant effects were further analyzed by Bonferroni post hoc. \*P < .05 versus control; †P < .05 versus 3 days, n = 5–11 per group.

establish the response to volume load in the isolated non-working heart.

Significant improvements of  $dp/dt_{max}$  and  $dp/dt_{min}$  were recorded in unloaded hearts at 30 days as compared with control hearts (Figure 2, A and B). A significant shift of the curves indicated enhanced contractile function for volumes between 0 and 90  $\mu$ L (Figure 2). For higher volumes, the contractile capacity of unloaded hearts at 30 days worsened (data not shown).

Maximal developed pressure was not significantly changed after a 30-day unloading period ( $96 \pm 18$  mm Hg) as compared with control ( $87 \pm 11$  mm Hg). According to the morphologic changes observed in unloaded hearts, maximal developed pressure was reached at different volume overloads. These were respectively  $188 \pm 48$   $\mu$ L for control hearts and  $127 \pm 31$   $\mu$ L after 30 days of unloading. The 2 values are significantly different ( $P = .033$ ).

**Chamber size and stiffness.** End-diastolic and end-systolic pressure–volume relationships were calculated according to Fletcher and associates.<sup>22</sup> Atrophic ventricular remodeling characteristically results in global chamber size reduction as assessed by diastolic pressure–volume relationship. After 30 days, unloaded hearts exhibited a significant decrease in end-diastolic volume, resulting in a significant leftward shift of the pressure–volume curve (Figure 3). An end-diastolic pressure of 30 mm Hg was found to correspond to a ventricular volume of  $167 \pm 8$   $\mu$ L (n = 5) and  $121 \pm 14$   $\mu$ L (n = 5), respectively, for control and 30-day unloaded hearts.

Ventricular chamber stiffness constant (k) obtained from the slope of the exponential end-diastolic volume–pressure curve was significantly increased from  $0.026 \pm 0.004$  in control to  $0.051 \pm 0.009$  in 30-day unloaded hearts ( $P < .001$ ).

## DISCUSSION

The current study investigated macroscopic and microscopic morphologic changes occurring in normal hearts

unloaded over a 90-day period and the impact these morphologic changes had on myocardial function. We found macroscopic and cellular atrophy to occur mainly during the initial 30 days of unloading. The alterations appeared to remain stable up to 90 days. Thus, atrophy seemed installed after 30 days of ventricular unloading. We also demonstrate that during this period of complete unloading, in the setting of atrophy contractile function remained intact.

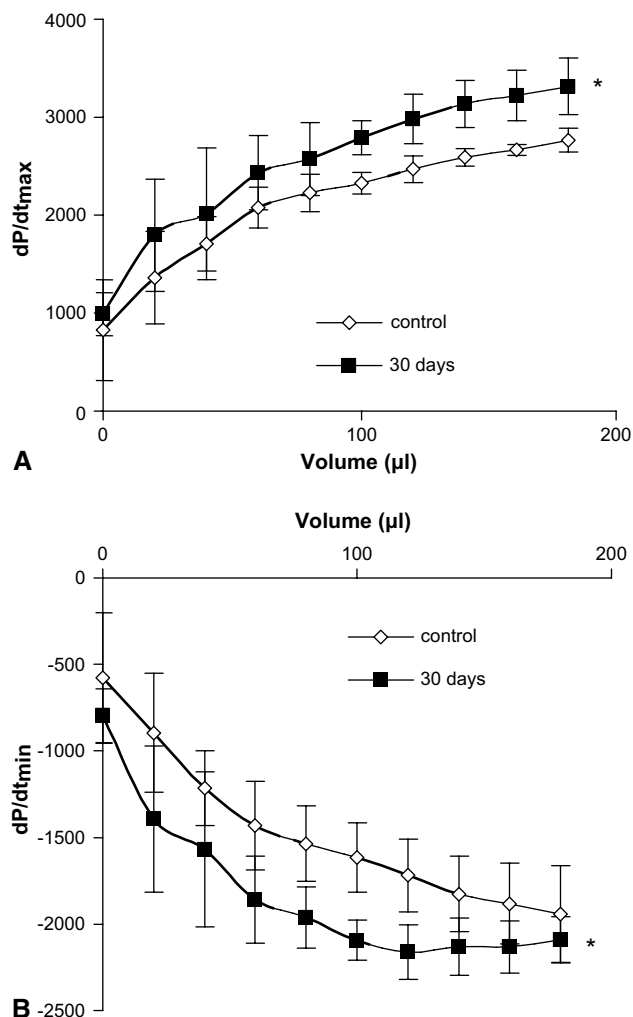
Reverse remodeling is believed to occur in unloaded failing hearts, and several studies have now confirmed its potential for partial functional recovery.<sup>1–3</sup> Hypertrophy, which is a typical feature of the remodeled failing ventricle, may clearly reverse if the heart is adequately unloaded. This was already demonstrated in echocardiographic studies observing patients after the replacement of a stenotic aortic valve<sup>23</sup> and was also observed in failing hearts supported by a VAD.<sup>24</sup> Reverse remodeling was reproduced in animal models of ischemic and overload-induced hearts<sup>13,17</sup>; indeed, it was shown that both myocyte size and shape return to almost normal in unloaded hearts. A better understanding of remodeling and the mechanisms underlying it may help to encourage the use of VADs as a bridge to recovery.

Whether atrophic changes limit functional recovery of failing hearts unloaded over a prolonged period of time has not been clearly answered. In fact, some authors have shown, at the cellular level, a reduction of contractile function.<sup>25</sup> Using isolated cardiomyocytes from unloaded normal rat hearts, these authors showed a time-dependent reduction in parameters of contractile function. On the other hand, Welsh,<sup>26</sup> Klotz,<sup>27</sup> and their associates showed that sarcomeric function is preserved despite clear morphologic atrophic changes. Our data confirm the later results inasmuch as we report a preserved or even improved contractile function in normal hearts completely unloaded over 1 month. Indeed, not only was maximal developed pressure preserved as compared with normal loaded hearts but, more important, contractility ( $dp/dt_{max}$ ) and relaxation

TABLE 2. Microscopic characterizations of the LV atrophy according to stereology principle

Parameter	Control	3 days	8 days	30 days	90 days
Total No. of myocyte nuclei in the ventricles ( $\times 10^6$ )	64.7 ± 5.1	60.6 ± 8.1	33.2 ± 2.3*	22.6 ± 3.7*	21.6 ± 3.1*
No. of myocyte nuclei per $\mu$ m <sup>3</sup>	8.0 ± 0.5	8.7 ± 0.1	6.9 ± 0.2	6.8 ± 1.0	7.0 ± 0.5
Myocyte volume per myocyte nucleus ( $\mu$ m <sup>3</sup> $\times 10^3$ )	9.7 ± 0.6	8.5 ± 0.9	9.3 ± 0.2	9.6 ± 0.1	9.5 ± 0.9

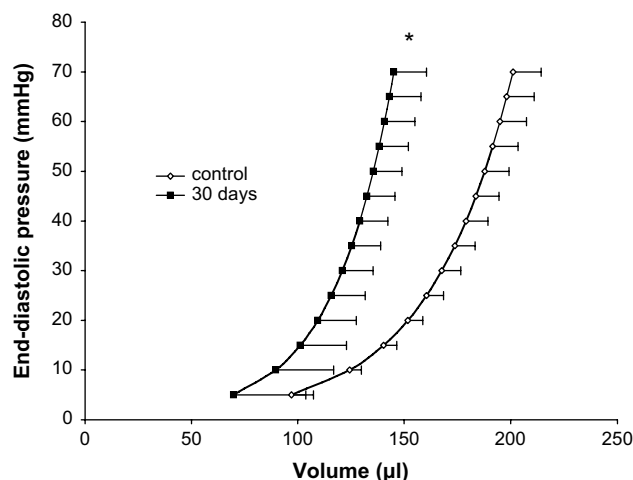
Stereologic analysis. \*P < .05 versus control. LV, Left ventricular.



**FIGURE 2.** Cardiac contractile function investigated in isolated hearts through recording of pressure response to intraventricular volume increases. Significant improvements of dP/dt<sub>max</sub> and dP/dt<sub>min</sub> were recorded in 30-day unloaded hearts as compared with control hearts (n = 3–5) (\*P < .05).

(dP/dt<sub>min</sub>) improved. However, owing to the limitations of the model we used to measure myocardial function, maintenance of contractility despite loss of muscle mass does not exclude a compromised capacity of the atrophic heart to support a systemic circulation.

Several authors have reported fibrosis occurring in hearts assisted with a VAD. However, the increase in collagen content has been questioned by others who reported on a reduction in collagen content after ventricular unloading.<sup>13,24,28</sup> A possible reason for the discrepancy regarding collagen content may be the technique used to analyze it. In these studies, the degree of atrophy was analyzed in a semiquantitative way, allowing for false interpretations inasmuch as the reference volume was not taken into account. Stereologic analysis eliminates this bias and, using this approach, Kinoshita and coworkers<sup>29</sup> showed that both myocyte and interstitial



**FIGURE 3.** End-diastolic pressure–volume relation in 30-day unloaded hearts demonstrating a significant leftward shift of the pressure–volume curve (\*P < .05) versus control (n = 3–5).

tissue volumes are reduced after 1 month of mechanical unloading with a VAD system in a large animal model. Using the same technique, we also demonstrated that atrophic changes occur in both the myocyte and interstitial compartments, although in different proportions. We not only confirmed the rapid onset of global ventricular atrophy but, more important, we showed that there is less of an active fibrosis in unloaded tissue. Instead, there is a disproportionate atrophic remodeling of the interstitial tissue compared with the cardiomyocyte compartment, accounting for a relative increase of the interstitial compartment. Indeed, during the first week of unloading, the proportion of interstitial tissue within the entire ventricles increased. Interestingly, this proportion remained constant over a 3-month period of time and, as previously mentioned, could not be correlated with functional alterations. The implications of these changes remain to be clarified. According to previous reports,<sup>30</sup> the increase in collagen content and especially the increase in collagen cross-linking may explain the augmented stiffness we observed in our study.

The observations in unloaded normal hearts may not directly reflect situations involving failing hearts, wherein the remodeling process is already initiated. Importantly, we<sup>17</sup> demonstrated previously that a prolonged progression to heart failure negatively influences the chance for functional recovery. Another limitation is that the evaluation of functional parameters such as hemodynamic data cannot be generated in this Langendorff approach. Because of the significant reduction in LV volume, it can be assumed that cardiac output would be decreased after unloading. Similarly, the Langendorff ex vivo analysis does not allow long-term functional analysis or conclusions regarding the sustainability of ventricular function. In addition, the heart transplantation model does not allow for partial unloading



or reloading of the transplanted heart. These two approaches are worth being evaluated with a working heart Langendorff model in which pressure–volume loops could provide cardiac output data and further LV function analyses including LV elastance and preload recruitable stroke work. Finally, denervation of the transplanted heart may also play a role in the atrophic remodeling process and cannot be considered with this model. Nevertheless, the heterotopic transplantation model is a convenient model that allows us to gain insights into the complexity of the remodeling process during chronic unloading.

In conclusion, since atrophic changes induced by unloading may not correlate with a decreased contractile function, our results encourage further investigations to improve the success rates of “bridge-to-recovery” programs.

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