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# Human Ventricular Unloading Induces Cardiomyocyte Proliferation

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## ABSTRACT

**BACKGROUND** The adult mammalian heart is incapable of meaningful regeneration after substantial cardiomyocyte loss, primarily due to the inability of adult cardiomyocytes to divide. Our group recently showed that mitochondria-mediated oxidative DNA damage is an important regulator of postnatal cardiomyocyte cell cycle arrest. However, it is not known whether mechanical load also plays a role in this process. We reasoned that the postnatal physiological increase in mechanical load contributes to the increase in mitochondrial content, with subsequent activation of DNA damage response (DDR) and permanent cell cycle arrest of cardiomyocytes.

**OBJECTIVES** The purpose of this study was to test the effect of mechanical unloading on mitochondrial mass, DDR, and cardiomyocyte proliferation.

**METHODS** We examined the effect of human ventricular unloading after implantation of left ventricular assist devices (LVADs) on mitochondrial content, DDR, and cardiomyocyte proliferation in 10 matched left ventricular samples collected at the time of LVAD implantation (pre-LVAD) and at the time of explantation (post-LVAD).

**RESULTS** We found that post-LVAD hearts showed up to a 60% decrease in mitochondrial content and up to a 45% decrease in cardiomyocyte size compared with pre-LVAD hearts. Moreover, we quantified cardiomyocyte nuclear foci of phosphorylated ataxia telangiectasia mutated protein, an upstream regulator of the DDR pathway, and we found a significant decrease in the number of nuclear phosphorylated ataxia telangiectasia mutated foci in the post-LVAD hearts. Finally, we examined cardiomyocyte mitosis and cytokinesis and found a statistically significant increase in both phosphorylated histone H3–positive, and Aurora B–positive cardiomyocytes in the post-LVAD hearts. Importantly, these results were driven by statistical significance in hearts exposed to longer durations of mechanical unloading.

**CONCLUSIONS** Prolonged mechanical unloading induces adult human cardiomyocyte proliferation, possibly through prevention of mitochondria-mediated activation of DDR. (J Am Coll Cardiol 2015;65:892–900) © 2015 by the American College of Cardiology Foundation.

Although modest, but measurable, cardiomyocyte turnover occurs in the adult heart (1,2), it is insufficient for the restoration of contractile function after substantial cardiomyocyte loss. In patients with heart failure, persistent pressure or volume overload results in progression of the underlying cardiomyopathy (3,4). Although this cardiac remodeling can be slowed or sometimes reversed by intense pharmacological therapy, this process is often progressive (5). In advanced heart failure patients, left ventricular assist devices (LVADs) result in improved cardiac output, systemic perfusion, and end-organ function (6,7), which have led to an exponential increase in their implantation over the past decade (8,9). Intriguingly, myocardial recovery allowing for LVAD explantation has been reported in small subsets of patients (10–12)

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and is thought to result from functional recovery of viable myocardium due to a combination of ventricular unloading and pharmacological therapy (13).

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Our group recently showed that activation of the DNA damage response (DDR) is an important mechanism of cell cycle arrest in postnatal mammalian cardiomyocytes (14). We showed that the buildup of mitochondrial mass postnatally results in increased reactive oxygen species (ROS) production, oxidative DNA damage, and activation of DDR. Although the relative hyperoxemia of the postnatal heart plays an important role in up-regulation of oxidative metabolism, increased mechanical load is also known to activate cardiac mitochondrial biogenesis (15). We therefore reasoned that mechanical unloading might reverse the metabolic cascade that results in cell cycle arrest of cardiomyocytes. In this respect, human LVAD hearts provide the unique opportunity to perform histological analysis in the same patient in 2 drastically variable physiological states. We conducted this study to test the effect of mechanical unloading on mitochondrial mass, DDR, and cardiomyocyte proliferation in patients who received LVADs.

## METHODS

**PATIENT SAMPLES.** Human heart tissue samples were obtained from patients with advanced heart failure after informed consent under 2 overlapping institutional review board protocols approved by the UT Southwestern Medical Center Clinical Institutional Review Board Committee (Institutional Review Board #STU 092010-193 and #STU 092010-093). The patients had been referred to the UT Southwestern Medical Center Heart Failure, Ventricular Assist Device & Heart Transplant Program for consideration of either implantation of an LVAD and/or a heart transplantation.

Paired heart tissue samples were obtained from each patient: first at the time of LVAD implantation and again at the time of heart transplantation. Pre-LVAD samples were acquired from the left ventricular apex, whereas the post-LVAD samples were obtained from the lateral wall of the left ventricle. Once the left ventricular tissue was removed from the patient, the tissue was either fixed for 48 h in 10% formalin or snap-frozen in liquid nitrogen. The fixed tissue samples were submitted to the UT Southwestern Medical Center Cardiovascular Histological Laboratory for paraffin embedding and processing for various immunohistological studies.

**MITOCHONDRIAL DNA QUANTIFICATION BY REAL-TIME POLYMERASE CHAIN REACTION.** For mitochondrial DNA (mtDNA) quantification, DNA was extracted and purified from tissue samples with proteinase K digestion and subsequent phenol/chloroform extraction. mtDNA was quantified with real-time polymerase chain reaction with the following primers: mtDNA F: CTAAATAGCCCACACGTTCCC; R: AGAGCTCC CGTGAGTGGTTA (targeting a relatively stable site in mitochondrial DNA minimal arc [16]), and nuclear DNA F: GCTGGGTAGCTCTAAA CAATGTATTCA; R: CCATGTACTAACAAATGTC TAAAATGGT (targeting single-copy nuclear DNA within the beta-2M gene [16]), using SYBR Green PCR Master Mix and the 7000 Sequence Detection System (Applied Biosystems, Foster City, California). The relative mtDNA copy number was calculated from the ratio of mtDNA copies to nuclear DNA copies per gram of tissue. The relative fold change was then calculated using the $\Delta\Delta C_T$ method.

**PROTEIN EXTRACTION FROM HEART TISSUE AND WESTERN BLOTTING.** Whole-cell extracts from human heart samples were prepared as described previously (14). Briefly, samples were homogenized in radioimmunoprecipitation assay buffer using a handheld homogenizer (Thermo Fisher Scientific, Waltham, Massachusetts) on ice for 30 min. Cell extracts were centrifuged at 14,000 rpm for 30 min at 4°C to remove insoluble material. Radioimmunoprecipitation assay buffer contained phenylmethylsulfonyl fluoride, aprotinin (1 μg/ml), leupeptin (1 μg/ml), pepstatin A (1 μg/ml), sodium fluoride (150 mM), and sodium metavandate (1 mM). Aliquots containing 200 μg protein were resolved by 8% sodium dodecylsulfate-polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membrane at 30 V at 4°C overnight. Membranes were blocked with 5% milk in Tris-buffered saline-0.1% Tween 20) at room temperature for 20 min and incubated with different antibodies in 5% milk in Tris-buffered saline-0.1% Tween 20 at 4°C overnight. Membranes were subsequently washed 3 times for 5 min each with Tris-buffered saline-0.1% Tween 20, and then incubated with horseradish peroxidase-conjugated secondary antibodies (anti-mouse/rabbit/goat) in 5% milk for 2 h at room temperature. The primary antibodies used for Western blotting were as follows: phosphorylated ataxia telangiectasia mutated (pATM) protein; 10H11.E12) (sc-47732, mouse, 1:500, Santa Cruz Biotechnology, Dallas, Texas) and cardiac troponin T (13-11, mouse, 1:10,000, Thermo Fisher Scientific, Richardson, Texas). Quantification analysis of the Western blot signal was done using ImageJ (National Institutes of Health, Bethesda, Maryland).

**ABBREVIATIONS AND ACRONYMS**

**DDR** = DNA damage response

**LVAD** = left ventricular assist device

**mtDNA** = mitochondrial DNA

**pATM** = phosphorylated ataxia telangiectasia mutated protein

**PBS** = phosphate-buffered saline

**pH3** = phosphorylated histone H3

**ROS** = reactive oxygen species