

Taking a Load Off Nuclear Remodeling After Mechanically Supporting the Failing Human Heart

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The heart adapts exquisitely to changing mechanical loads by changing its size, shape, stiffness, contractility, and electric responsiveness to meet new demands. In recent years we have learned a great deal about how increased load activates signaling pathways that lead to changes in expression of regulatory molecules, contractile proteins, extracellular matrix components, and ion channels.¹ Although these pathways are initially adaptive, they can become deleterious when activated to excess, contributing to heart failure. It is less well recognized, however, that human cardiomyocytes initiate cell cycle activity in response to mechanical stress. For example, Adler and Friedburg showed more than 2 decades ago that mechanical loading is associated with increased nuclear ploidy (DNA content per nucleus) and with increased nuclear number in human cardiomyocytes.² Furthermore, several groups have identified increased cardiomyocyte DNA synthesis in disease states.³ Together, these data suggest that, in response to mechanical stress, differentiated cardiomyocytes can replicate their DNA without undergoing karyokinesis (nuclear division) or cytokinesis (cell division), resulting in a higher DNA content per cell.

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In principle, increasing a cell's DNA content is associated with increased cell size and metabolic output without the necessity of rearranging the cytoskeleton and extracellular attachments (as happens in mitosis).⁴ In addition, polyploid cells may be more resistant to DNA damage, probably because they have many copies of every gene and because they need not segregate their chromosomes during mitosis.⁴ Thus, increasing DNA content and cell size may be an advantageous strategy for tissues that are terminally differentiated yet must continue to grow—for example, in the heart exposed to increased mechanical load. In this issue of *Circulation*, Wohlschläger et al⁵ bring a new perspective to the dynamics of DNA content in human cardiomyocytes by showing a reduction of the polyploid cardiomyocyte DNA

content in failing hearts during unloading with left ventricular assist devices (LVADs).

Left ventricular assist devices are increasingly used to bridge advanced heart failure patients to transplantation. The LVAD procedure offers a remarkable window into adaptation of the human heart because tissue is obtained before unloading, from the apical plug removed for conduit attachment, and after unloading, from the explanted heart. Seminal studies have shown that LVAD unloading causes regression of hypertrophy at the whole heart and single myocyte level, accompanied by reduction in interstitial fibrosis.^{6–8} Isolated myocytes show enhanced contraction and relaxation dynamics, improved calcium handling and sarcoplasmic reticulum function, and improved β -adrenergic signal transduction.^{6,9} Recovery of the heart is sufficient in some instances to permit weaning from the LVAD without heart transplantation.¹⁰

Wohlschläger and colleagues⁵ studied paired samples of human myocardium from 23 heart failure patients before and after LVAD implantation, comparing their findings with those from 5 healthy control hearts. The LVADs were in place for a median of 146 days before transplantation (range, 17 to 426 days). The myocardial samples were subjected to a battery of “molecular morphometry” analyses, including Feulgen staining to quantify DNA content, fluorescence in situ hybridization to assess chromosome number, and more standard histopathologic evaluations to assess nuclear size and number per myocyte. They confirmed previous findings showing that myocytes in failing hearts had markedly increased DNA content. Impressively, cardiomyocytes with 32C DNA content (where C equals a haploid set of chromosomes) were identified in failing hearts.

Wohlschläger et al report that long-term LVAD support induced significant changes in cardiomyocyte nuclear makeup. After unloading, cardiomyocyte nuclei were smaller, and they showed a 30% decrease in their median DNA content, from 6.8C to 4.7C, though still higher than control hearts at 3.0C. This correlated with a reduction in chromosome 8 fluorescence in situ hybridization signals per nucleus as well. A surprising finding was that the percentage of cardiomyocytes with 2 or more nuclei increased after LVAD implantation, from a median of 4.5% to 10.0%, whereas healthy control hearts had \approx 2% of myocytes with 2 or more nuclei. Aficionados of cardiac structure will note that this is a low baseline rate of multinucleation. Studies using dispersed cells or serial sectioning generally place multinucleation rates in human cardiomyocytes at \approx 25%.^{11,12} In addition, 1 prior study reported no difference in the percentage of mononucleated and binucleated cardiomyocytes between failing hearts without LVAD support and those after a mean of

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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(*Circulation*. 2010;121:957-959.)

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Circulation is available at <http://circ.ahajournals.org>
DOI: 10.1161/CIR.0b013e3181d61b63

75 days of LVAD support.⁷ These and other differences may be related to the authors' use of single histological sections, which will miss some of the nuclei in multinucleated cells. Along these lines, the apparent increase in nuclear number may be a morphometric artifact, because regression of hypertrophy will bring nuclei closer together, increasing their likelihood of detecting more than 1. Despite the apparent increase in nuclear number, the authors found that total DNA content per cardiomyocyte significantly decreased after LVAD unloading. This resulted from significant decreases in the percentage of cardiomyocytes with 8C or greater DNA contents, and an increase in the percentage with 2C (ie, diploid) and 4C DNA content.

Taking these results together, Wohlschläger and colleagues demonstrate that mechanical unloading results in reduced cardiomyocyte DNA content in human hearts. How does this happen? If these data apply to the individual cardiomyocyte, a reduction in DNA content may be due to cellular division of polyploid cardiomyocytes without prior DNA replication. (Division of the nucleus without cytokinesis will change only nuclear ploidy, not cellular DNA content, and hence karyokinesis cannot explain the observations.) On the other hand, it is important to remember that these data are derived from populations of cells, not individual cells studied longitudinally, and this raises additional possibilities. For example, unloading could induce death in cardiomyocytes with high DNA content, thus pushing mean DNA content downward. Tea et al have shown that regression of cardiac hypertrophy induced by antihypertensive drugs is accompanied by a wave of cardiomyocyte apoptosis in rats.¹³ A third mechanism involves the generation of new cardiomyocytes from undifferentiated stem or progenitor cells, which presumably would be biased toward a diploid DNA content. The current data do not permit distinction of these 3 possibilities.

Several recent publications offer insights into possible mechanisms. Bergmann and colleagues recently published a fascinating study of turnover of nuclear DNA in normal human hearts.¹⁴ By studying the pulse-chase of ¹⁴C resulting from atmospheric testing of nuclear weapons, the authors estimated that human cardiomyocytes turn over at a rate of 1% per year at age 20, declining to 0.5% per year by age 50. Performing a similar analysis before and after the use of an LVAD would help to distinguish death of polyploid cells (which should not change net ¹⁴C activity) from birth of new cardiomyocytes (which should dilute the Cold War ¹⁴C with contemporary levels). Although adult cardiomyocytes are traditionally regarded as nondividing cells, several lines of evidence suggest that they may be capable of low-level cell division. Bersell et al recently showed that adult mouse cardiomyocytes can be induced to divide in response to neuregulin signaling,¹⁵ albeit at low levels, and pathological evaluations of human hearts have reported nuclear division, and possible cellular division, of cardiomyocytes in the peri-infarct region.¹⁶ Finally, several lines of evidence now suggest that there may be stem cells in the adult heart capable of cardiomyocyte repopulation. Hsieh et al used a genetic pulse-chase strategy to label preexisting cardiomyocytes.¹⁷ They showed that, after myocardial infarction or pressure overload, these preexisting cells became diluted by nonla-

beled cells, consistent with generation of new myocytes from stem cells. It is interesting that, whatever the mechanism underlying the changes in ploidy in the report by Wohlschläger et al, it appears to be relatively rapid, given that they occur in as few as 17 days of LVAD support.

What are the consequences of changes in cardiomyocyte ploidy? It seems reasonable that increases in DNA copy number would result in the long-term increases in gene expression and cellular protein levels that are characteristic of hypertrophy. If so, increased ploidy may play a deterministic role in the heart either by initiating or by maintaining the hypertrophic state. Conversely, reductions in ploidy as observed by Wohlschläger and colleagues may be necessary and/or sufficient for the regression of hypertrophy induced by LVADs. It would be interesting to develop a model wherein cardiomyocyte ploidy could be dialed upward or downward, independently of loading, and test the impact of these changes on baseline cardiac mass and stress responsiveness.

These studies indicate that the "end-stage" failing heart may not be so end-stage after all. The salutary effects of unloading the heart with an LVAD are undisputed, affecting the heart's size, shape, passive compliance, excitation-contraction coupling, myofilament composition, and now its DNA content and nuclear distribution. Can this mechanical benefit be otherwise triggered? Ventricular unloading must control signaling pathways where changes in force are transduced to changes in neuroendocrine signaling, gene expression, and DNA metabolism. If we can understand and control these pathways, it should be possible to derive many of the benefits of LVADs without the need for surgical device implantation.

Disclosures

Dr Kühn is the founder of CardioHeal. Dr Murry reports no conflicts.

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KEY WORDS: Editorials ■ DNA ■ remodeling ■ regeneration ■ stem cells