

Reproducibility of left atrial ablation with high-intensity focused ultrasound energy in a calf model

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Objective: Achieving transmural tissue ablation might be necessary for successful treatment of atrial fibrillation. The purpose of this study was to evaluate the reproducibility of transmural left atrial ablation using a high-intensity focused ultrasound energy system in a calf model.

Methods: Nine heparinized bovines underwent a beating-heart left atrial ablation with a single application of the high-intensity focused ultrasound device. All animals were acutely killed, and the left atrium was fixed in formalin. Protocolized histological sections (5 μ m) were obtained throughout each lesion and prepared with Masson trichrome and hematoxylin and eosin staining. Measurements were performed on a total of 359 slides from the 9 lesions. In addition, fresh left atrial tissues from 18 unused human donor hearts that did not meet the criteria for cardiac transplantation were measured at the site where the high-intensity focused ultrasound device is normally applied.

Results: Calf left atrial thickness ranged between 2.5 and 20.1 mm, with a mean of 9.10 mm. High-intensity focused ultrasound ablation consistently produced a 100% transmural lesion in left atrial thickness up to 6 mm. In addition, a transmural lesion was observed in 91% of tissues that were up to 10 mm thick and in 85% that were up to 15 mm thick. Human left atrial thickness ranged between 1.2 to 6 mm, with a mean of 3.7 mm.

Conclusions: Calf left atrial thickness in this study was greater than human left atrial thickness. Human left atrial thickness is generally less than 6 mm, and in this range high-intensity focused ultrasound ablation achieved 100% transmurality. These histological results might correlate with a high success rate of atrial fibrillation ablation by using the high-intensity focused ultrasound system. (*J Thorac Cardiovasc Surg* 2010;140:1381-7)

Supplemental material is available online.

Atrial fibrillation (AF) is present in approximately 9% of patients older than 70 years and has been recognized as an independent risk factor for cardiovascular morbidity and mortality.¹ Reported freedom from AF with medical management and catheter ablation techniques is inferior to the cut-and-sew Cox maze III procedure, which con-

tinues to be the gold standard for the treatment of AF, with 92% to 97% cure rates at 10 years of follow-up.² However, this procedure has not been widely adopted because of its technical complexity and because of the required additional cardiopulmonary bypass time. The identification of trigger zones for AF,³ specifically around the pulmonary vein orifices, has led to the development of simpler, less invasive procedures using different energy sources. Several different energy sources applied endocardially, epicardially, or both have been used for this purpose with variable results. A common goal when developing new technologies for atrial ablation has been to create transmural lesions similar to what is obtained with the cut-and-sew Cox maze procedure. Some of the challenges that these new technologies need to overcome include the ability to ensure transmurality without damage of the surrounding structures in the setting of variations in thickness, fat content, and the cooling effect of atrial blood flow. High-intensity focused ultrasound (HIFU) is a young technology in the armamentarium for AF ablation. Clinical trials performed in Europe⁴ and the United States⁵ with HIFU in patients with AF undergoing concomitant cardiac surgery have reported a rate of freedom from AF of 85% at 18 months of follow-up. However, a histological analysis demonstrating the presence of transmural

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Abbreviations and Acronyms

AF	= atrial fibrillation
HIFU	= high-intensity focused ultrasound
LA	= left atrium

lesions with HIFU had not yet been published. The purpose of this study was to assess the safety and reproducibility of transmural left atrial (LA) ablation with the Epicor LP HIFU system (St Jude Medical, St Paul, Minn) in a calf model.

MATERIALS AND METHODS**Device Description**

The Epicor Cardiac Ablation System has been previously described.⁴ Briefly, the system consists of the Ablation Control System generator, a family of disposable ablation devices, and a set of accessories. The Ablation Control System is a microprocessor-based unit that enables acoustic power to be delivered to the heart from ultrasound transducers. The UltraCinch LP device is an array of multiple (7–13) ultrasound transducers (cells) that is used to produce a transmural circumferential LA lesion, isolating the posterior LA and all 4 pulmonary vein orifices. An additional tool, the UltraWand LP, is a handheld ablation device with only 2 transducers for the epicardial creation of additional linear lesions, such as the line to the mitral annulus. The UltraWand LP was used in this model to assess the effect of HIFU energy applied over coronary vessels.

Animal Studies

The study was conducted at Duke University under approval of the Institutional Animal Care and Use Committee. All animals received humane care in compliance with the “Guide for the care and use of laboratory animals.” Nine calves with a weight range of 63 to 114 kg were selected for the study.

System Characteristics

The transducers comprising the UltraCinch LP are positioned on the epicardium but separated from direct contact with the cardiac tissue by a thin perforated membrane. Room temperature normal saline is circulated between the membrane and the transducers during ablation to enhance acoustic coupling and cooling. Each transducer (10 × 15 mm) is designed to deliver HIFU energy up to a distance of 10 mm from its surface. Beyond the focal point, the energy dissipates within the LA cavity without exposing the surrounding structures to potential collateral damage. The proprietary UltraCinch LP algorithm generated by the Ablation Control System uses a combination of frequency, power, and duration at which the transducers are activated and powered (from 3.8–6.4 MHz and from 15–130 W) to generate 3 sequential stages of ablation. The ablation process begins with the deep ablation stage, during which the energy is deposited distally from the transducer in the subendocardial zone, followed by the intermediate stage in which energy is deposited in the midmyocardial layer, followed by the surface stage for epicardial energy deposition. During the first 2 stages, each transducer is activated sequentially, and the energy is pulsed, whereas during the third stage, transducers are staged 3 at a time with continuous-wave HIFU to eliminate any residual gap within the epicardium. The lesion is thus built up from the endocardium back to the epicardium and is complete within approximately 10 minutes. The UltraWand LP is activated in a similar fashion but uses a single stage for the creation of linear lesions during a shorter 80-second algorithm that was designed for a handheld device.

Surgical Technique

The animals were anesthetized with isoflurane, and a median sternotomy was performed. The surgical ablation was executed as previously described by Ninet and colleagues.⁴ The pericardium was opened, and the pericardial reflections around both the superior vena cava and inferior vena cava were dissected free to gain access to both the transverse and oblique sinuses. A specially designed introducer sizer was passed behind the superior vena cava into the transverse sinus and guided into the oblique sinus and beneath the inferior vena cava, thereby completely encircling all 4 pulmonary veins. The graduated introducer sizer was used to measure the circumference of the LA to select the proper size of UltraCinch LP device. The introducer was subsequently used to guide the UltraCinch LP around the LA. The introducer was then removed, and the 2 ends of the UltraCinch LP device were approximated with tourniquets placed on the appropriate sutures at each end to snug the device securely around the LA (Figure 1, A). A 7- or 8-cell Epicor UltraCinch LP device was used in all 7 cases. A bolus of heparin (100 IU/kg) was administered before the ablation cycle start, and a continuous saline infusion was set by gravity with a bag height of 35 inches, which yields approximately 1.5 mL/min per cell of irrigation into the membranes overlying the transducer cells. The ablation cycle was then initiated and progressed automatically until the UltraCinch LP ablation had been completed. Physiologic data were recorded during the ablation run. An additional lesion placed over the coronary sinus was also created epicardially with the UltraWand LP (Figure 1, B). A continuous saline infusion was maintained at a rate of 120 mL/h during the UltraWand LP ablation cycle.

Tissue Processing

All animals were killed at the end of the procedure, and the heart was immediately explanted for macroscopic examination. The LA and additional cardiac tissue surrounding the UltraCinch LP and UltraWand LP lesions was widely resected en block. Endocardial and epicardial surfaces

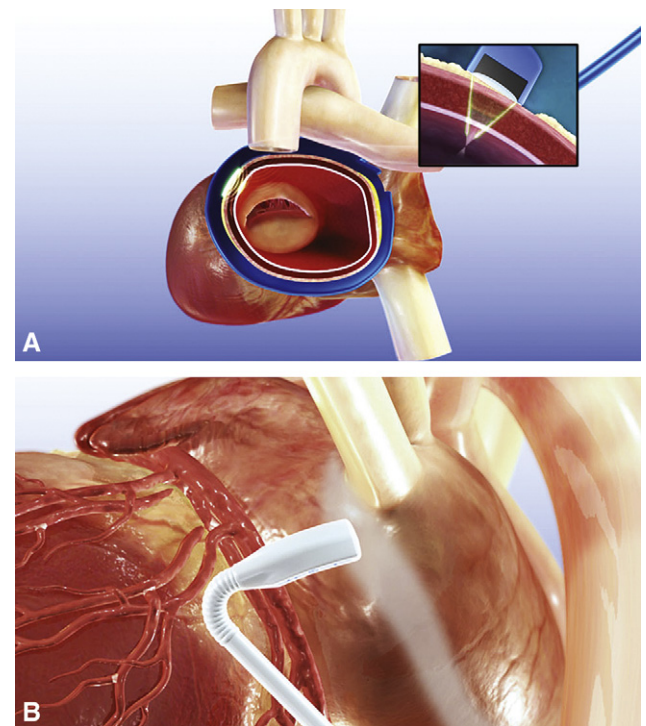


FIGURE 1. Sites of high-intensity focused ultrasound ablation. A, Position of the Epicor UltraCinch LP. B, Position of the Epicor UltraWand LP.

were examined for color, appearance, evidence of perforation or rupture, presence of intramural hemorrhage, presence of endocardial thrombus, integrity of the pulmonary vein wall, and the mitral valve apparatus. The resected tissue was then fixed in 10% formalin until sectioning. The fixed LA was radially sectioned into 8 blocks, approximately equivalent to the locations of the individual device cells (see Figure E1). The wand lesion was serially sectioned perpendicular to the coronary sinus. Five standard 5- μ m sections were prepared from each block, alternating Masson trichrome and hematoxylin and eosin staining, and additional sections were prepared in the same manner until each block was completely sectioned. All sections were examined, and measurements were taken from 2 separate sections of each block by using an ocular micrometer.

Measurements

Measurements were obtained from a total of 359 slides. Tissue thickness was measured at the site of the lesion. Lesion thickness was measured. In addition, fat and myocardial thickness, which composed the lesion, was recorded. Lastly, any gap of uninjured tissue was measured. Lesion transmural-ity was calculated by comparing lesion depth and total tissue thickness, as shown in Figure 2.

Human Studies

Human hearts that did not meet criteria for cardiac transplantation were procured after research consent was obtained from our local organ-procurement organization. Hearts were procured in standard fashion from donors in which the lungs were also not used for transplantation to allow for dissection and isolation of 2 to 3 cm of each pulmonary vein. The LA was separated from the left ventricle at the atrioventricular groove immediately above the mitral valve similar to the procurement of the bovine LAs. Measurements were made with a micrometer adjacent to but 2 mm central to each of the 4 pulmonary veins in the perceived location of the UltraCinch LP device. Three measurements were taken at each location and averaged. Data are presented as means \pm standard errors of the mean.

RESULTS

Animal Studies

Macroscopic examination. There was no evidence of perforation or rupture of the cardiac tissues subjected to ablation in any of the 9 animals. Gross examination of the UltraCinch LP application site revealed a wide, distinct,

circumferential lesion on the epicardium with correspond-ing linear tracts on the endocardial surface on inversion of the specimen, indicating transmural penetration of the lesion (Figure 3, A). Importantly, there was no evidence of LA endocardial injury distant to the lesion. No intramural hemorrhage or endocardial thrombus was identified in any of the 9 animals. The pulmonary vein wall and mitral valve apparatus demonstrated freedom from any injury in all 9 animals. Gross examination of the UltraWand LP application site did not show evidence of acute cavitation, perforation, thrombosis of epicardial coronary vessels, or other unin-tended trauma to the target tissue.

Microscopic examination. Microscopically, the type of injury caused by the Epicor UltraCinch LP device was char-acterized by a wedge-shaped lesion with a wide base at the epicardial surface that progressively narrowed to the endo-cardial surface. The histologic features include edema, nu-clear hyperchromasia, and contraction bands, which are typical of irreversible myocardial injury (Figure 3, B).

Calf total LA thickness for all 359 slides ranged between 2.5 and 20.1 mm, with a mean of 9.10 mm. Adipose tissue thickness at the lesion site ranged between 0 and 16 mm,

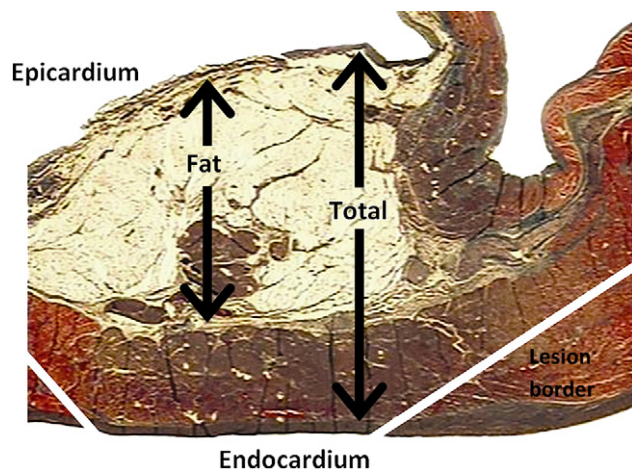


FIGURE 2. Histology of the high-intensity focused ultrasound lesion with total versus fat thickness measurement.

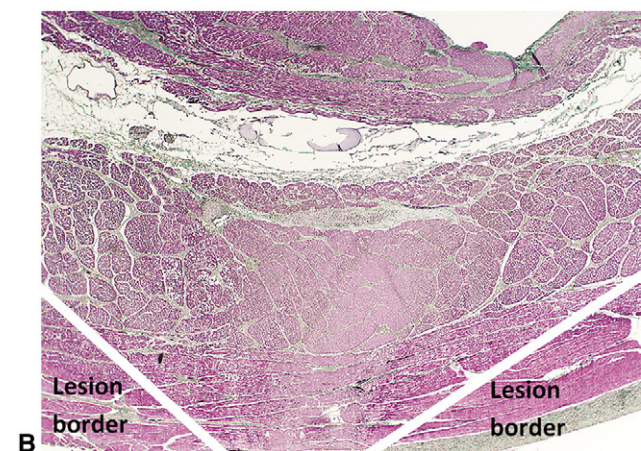
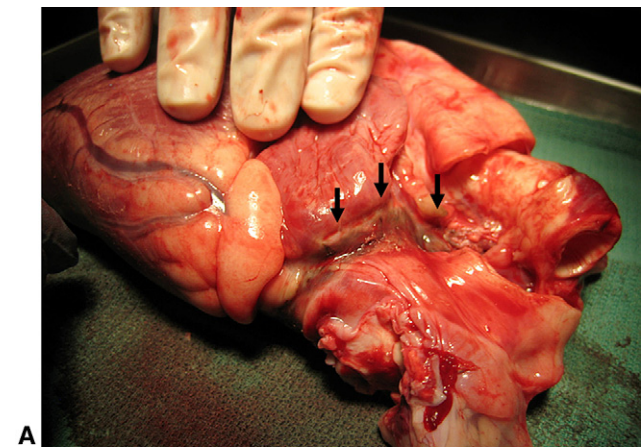


FIGURE 3. Characteristics of Epicor UltraCinch LP ablation. A, Macroscopic examination. B, Microscopic examination.

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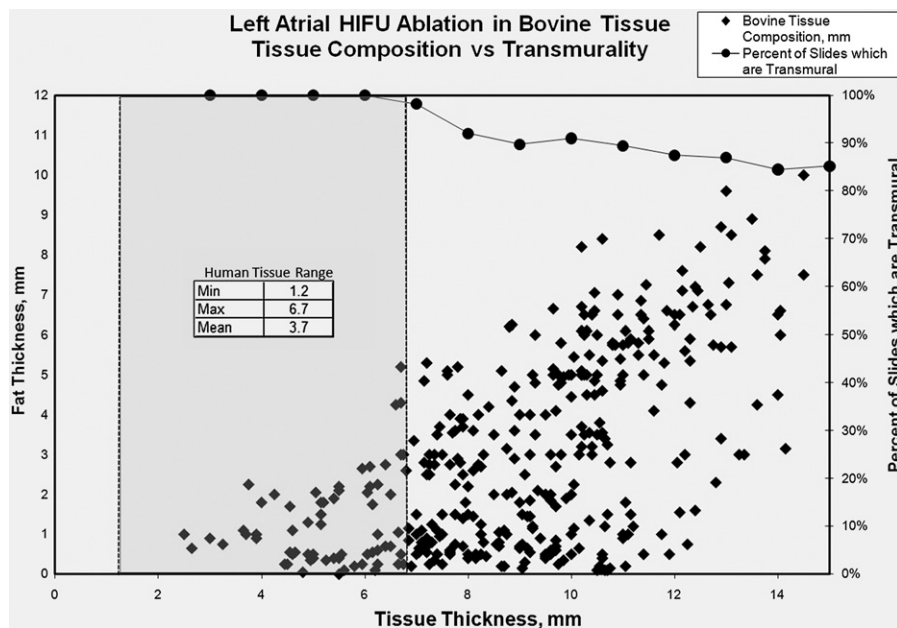


FIGURE 4. Percentage of transmural lesions in relation to distribution of total thickness and percentage of adipose tissue using the Epicor UltraCinch LP. *HIFU*, High-intensity focused ultrasound.

with a mean of 3.10 mm. The distribution of total thickness and percentage of adipose tissue for the entire cohort is represented in Figure 4. Seventy-one percent of the measured slides contained LA tissue that was 10 mm or less in total thickness, which is the greatest depth at which the HIFU beam is focused.

The Epicor UltraCinch LP consistently produced a 100% transmural lesion in LA thickness up to 6 mm, which includes adipose tissue of up to 2.7 mm. In addition, a transmural lesion was observed in 91% of tissues that were up to 10 mm thick and in 85% of tissues that were up to 15 mm thick (Figure 4). The greatest total tissue thickness that underwent a 100% transmural lesion was 14.5 mm. The greatest total adipose tissue that underwent a 100% transmural lesion was 9.6 mm. Nontransmural lesions were not located at a defined anatomic landmark in every animal nor was there a correlation between nontransmural lesions and the cell number within the Epicor UltraCinch LP. The amount of adipose tissue present on the target LA did not appear to correlate with lesion transmural. In contrast, the shape of the target tissue and the orientation of the HIFU lesion (the direction of the wedge-shaped lesion) seemed to play an important role in obtaining transmural (see Figure E2).

All nerve bundles identified within the HIFU beam appeared irreversibly injured independent of size or tissue depth (see Figure E3).

The Epicor UltraWand LP lesion was microscopically similar to the UltraCinch LP lesion. No thrombosis was seen inside the coronary sinus or circumflex artery over which the Epicor UltraWand LP was applied. However, there is some evidence of insult to smaller vessels that

cannot be fully characterized. The endothelial cell nuclei are rounded, enlarged, and protrude into the lumen; these are characteristic features of injured or reactive endothelial cells. There are no specific histologic features that allow differentiation between reversible versus irreversible endothelial cell injury. It should be noted that no injury to the circumflex artery or other nearby vessels has been observed in chronic human studies extending as far out as 18 months after the procedure.^{5,6}

Human Studies

To determine the relationship of the calf model to adult human LA thickness, we also measured LA thickness in 18 adult human unused donor hearts. The average age of hearts examined was 51.8 ± 4.6 years, and 28% had a history of cardiovascular disease. The mean human LA thickness was 3.7 mm, with a range of 1.2 mm to a maximum thickness of 6 mm.

DISCUSSION

AF is a significant cause of cardiovascular morbidity and mortality.¹ The success rate of medical therapy and catheter ablation remains suboptimal. The cut-and-sew Cox maze III surgical procedure has proved to be very effective but still carries a significant risk for complications and is technically challenging.² The development of new technologies to reproduce the outcomes obtained with the Cox maze III procedure but to minimize risks is an area of active investigation. Alternative energy sources used to treat AF include hypothermic sources (cryoablation) and hyperthermic sources (radiofrequency, microwave, laser, and ultrasound ablation).

Since Haissaguerre and colleagues³ identified the pulmonary vein orifices as the trigger zones for AF, a great interest has emerged to design ablation devices capable of isolating these trigger zones. The Epicor LP HIFU system has been designed to electrically isolate these areas in a safe and reproducible manner.

Although the necessity to achieve conduction block acutely is still a debatable topic, mature transmural lesions will always result in chronic electrical block. Accordingly, the only guarantee of long-term effectiveness is a fully transmural lesion. For instance, Melby and associates⁶ have shown that AF can conduct through gaps of 1 mm or greater in ablation lines. Furthermore, gaps along the lines of prior ablation have been identified in patients with unsuccessful surgical ablation.⁷ One of the end points of this study was to determine the presence of transmural lesions in the ablations created with the Epicor LP HIFU system. A single epicardial, beating-heart application of the Epicor UltraCinch LP device was 100% effective in producing a transmural lesion in calf atrial tissue up to 6 mm thick, which was the maximal thickness of measured adult human LA.

In comparison, animal studies with different energy sources have reported a wide range of lesion thickness in beating-heart models (Table 1).⁸⁻¹⁴ Cryoablation has produced transmural lesions in 25% to 100% of samples in beating-heart models with a mean LA thickness of 2.0 ± 0.3 mm.⁸ Transmurality with microwave ablation has only been achieved in 20% to 46% of samples in beating-heart animal models with a mean LA thickness of 2.62 ± 1.31 mm.⁹⁻¹¹ Only 1 study has reported transmural lesions with a laser energy source for surgical AF in beating hearts, which showed all lesions to be transmural in dogs, with an average tissue thickness of 3.62 ± 1.50 mm (range, 0.95–7.06 mm).¹² Bipolar radiofrequency clamp devices have been shown to create transmural lesions in 92% to 100% of samples in beating-heart animal models.^{13,14} However, the average tissue thickness was 4.2 ± 0.1 mm in the swine model¹³ and 1.9 ± 1.1 mm in the sheep model.¹⁴ In this study we tested the HIFU product in an animal model with much greater LA thickness relative to other beating-heart models and with only a single application, yet the experimental lesions in this study were deeper than those observed in the other models.

In our analysis of human LA thickness, we found that the average human LA measures 3.7 mm (range, 1.2–6 mm). Our results are similar to those of other anatomic studies aimed to define the human LA anatomy to provide a firm background for the development of AF ablation techniques. Cabrera and coworkers¹⁵ reported a mean LA thickness of 2.8 ± 1.1 mm (range, 1.5–4.2 mm) between the orifices of the left pulmonary veins and the os of the LA appendage in a total of 22 human hearts. Ho and colleagues¹⁶ found a mean LA thickness of 3.7 ± 0.6 mm (range, 2.9–4.5 mm) at the venoatrial junction of the left superior pulmonary vein in a total of 26 human hearts. Sánchez-Quintana and associates¹⁷ showed a mean LA thickness of 6.5 ± 2.5 mm (range, 2.8–12 mm) at the middle of the posterior atrial wall in a total of 15 cadavers. These results suggest that the LA thickness of the canine, swine, and sheep models in previous reports falls short of maximal adult human LA thickness. Thus these models might not be ideal for testing transmural lesions. In contrast, the calf model provides a tissue thickness much greater than human LA, as demonstrated by our findings.

Another advantage of the Epicor LP HIFU system is that epicardial fat dissection is not necessary to achieve transmural ablation. The average amount of adipose tissue in the 9 animals included in this study was 3.10 mm (range, 0–16 mm). As an example, a lesion of 20.2 mm total thickness with 16 mm of adipose tissue had a gap of only 0.55 mm of nonablated tissue after a single application of the Epicor UltraCinch LP device, which demonstrates the efficacy of HIFU to penetrate through fat. In contrast, Turek and coworkers¹⁸ reported that microwave energy has diminished penetration through adipose tissue. Assessment of adipose tissue penetration by using other energy sources (cryoablation, radiofrequency ablation, and laser ablation) has not been reported.

Some of the factors that might influence the depth of the ablation achieved with alternative energy sources are anatomy, tissue composition, device sizing and position, and saline flow rates. It did not appear that the amount of adipose tissue present on the target LA significantly contributed to the depth of the ablation in this model. However, the shape of the target tissue and the orientation of the HIFU beam seem to play an important role in obtaining transmural lesions.

TABLE 1. Transmurality in beating-heart models

Study	Energy source	Animal model	Mean LA thickness (mm)	Transmurality (%)
Doll and coworkers, ⁸ 2003	Cryoablation	Dog	2.0 ± 0.3	96
Van Brakel and coworkers, ⁹ 2004	Epicardial microwave	Dog	NS	33
Melby and coworkers, ¹⁰ 2006	Epicardial microwave	Pig	2.62 ± 1.31	46
Gaynor and coworkers, ¹¹ 2006	Epicardial microwave	Pig	2.88 ± 0.4	20
Williams and coworkers, ¹² 2006	Laser	Dog	3.62 ± 1.5	100
Melby and coworkers, ¹³ 2006	Bipolar RF	Pig	4.2 ± 0.1	100
Bugge and coworkers, ¹⁴ 2005	Bipolar RF	Sheep	1.9 ± 1.1	92
Villamizar and coworkers, 2010	HIFU	Calf	9.10 ± 2.6	84

LA, Left atrial; NS, nonspecified; RF, radiofrequency; HIFU, high-intensity focused ultrasound.

All animals underwent ablation with either a 7- or 8-cell Epicor UltraCinch LP device. Because each cell measures 1.0×1.5 cm, it is possible to create a significant gap interface between the cell surface and the epicardium when oversizing the circumference around the pulmonary vein orifices. On the other hand, undersizing might inhibit saline flow to cool off the Epicor UltraCinch LP cells and maintain their integrity throughout the ablation cycle. Thus appropriate sizing is important when using this epicardial technology.

Another end point of this study was to determine the presence of vascular injury and thrombotic complications in areas where the Epicor LP HIFU system is applied. No thrombotic complications were appreciated with the use of the Epicor LP HIFU system in this study. In comparison, unipolar radiofrequency ablation has been associated with thromboembolic complications,¹⁹ which appear to be absent with the use of bipolar radiofrequency devices.²⁰ There have also been reports of coronary artery stenosis with microwave technology.²¹ Although experience has shown that cryothermal energy appears to have no permanent effects on valvular tissue or the coronary sinus, experimental studies have shown late minimal hyperplasia of coronary arteries.²²⁻²⁴ There are no reports about the effect of laser ablation on the coronary vessels. The effect of HIFU ablation on the small vessels found within the HIFU beams appeared to be less destructive than has been observed with other energy sources, such as microwave ablation.¹⁸ The ability of HIFU to ablate tissue surrounding the coronary arteries without damaging the arteries themselves has been attributed to the protective cooling effect provided by the high blood flow through the coronary arteries and the absence of acoustic heating of the blood.²⁵

This study has some limitations that deserve discussion. First, it uses a calf model to investigate the ablative characteristics of a device designed to ablate human cardiac tissue. It is unclear how healthy calf atrial tissue compares with diseased human atria in patients with AF. Second, this study represents an acute examination of the injured tissue; some cells that appear dead might resurrect, and cells that appear viable might die later. Anatomy or dimensions of the scar at 1 to 3 months might appear different, and therefore we are planning more chronic studies. Third, conduction block was not evaluated. The calf LA thickness was considerably greater than the human LA thickness, and therefore gaps were anticipated. Also, calves have a left superior vena cava that traverses the site of the HIFU lesion, resulting in greater total tissue thickness and preventing transmural ablation at this site. Finally, acute conduction block might not correlate with late conduction block.

Despite these limitations, some assumptions could be made from the observed results. The average calf LA thickness in this study was 9.10 mm, and the range was between 2.5 and 20.1 mm. In contrast, the average LA in 18 human

hearts ranged between 1.2 and 6 mm, with a mean of 3.7 mm. HIFU ablation consistently produced a 100% transmural lesion in LA tissue thickness up to 6 mm. Human LA thickness is generally less than 6 mm, and in this range HIFU ablation should achieve transmural ablation.

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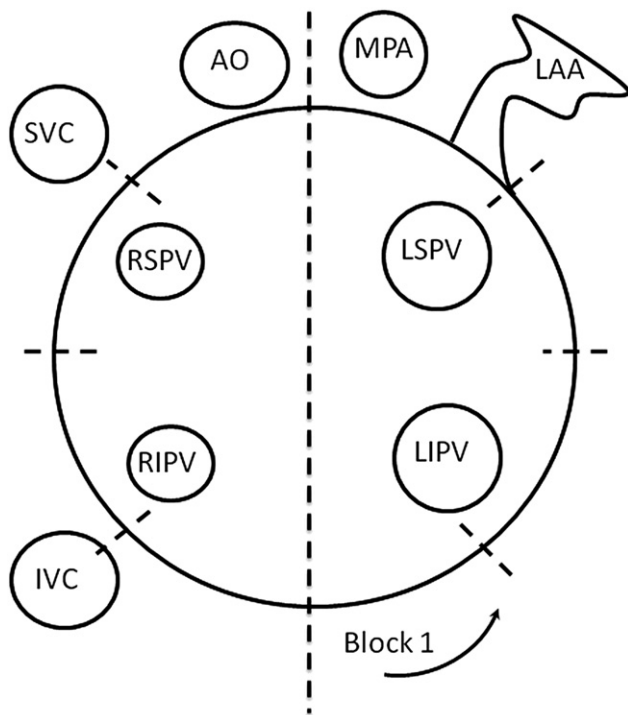


FIGURE E1. Representation of how the fixed left atrium was divided into 8 sections (blocks) along the high-intensity focused ultrasound lesion to obtain histology slides. *Ao*, Aorta; *IVC*, inferior vena cava; *LAA*, left atrial appendage; *LIPV*, left inferior pulmonary vein; *LSPV*, left superior pulmonary vein; *MPA*, main pulmonary artery; *RIPV*, right inferior pulmonary vein; *RSPV*, right superior pulmonary vein; *SVC*, superior vena cava.

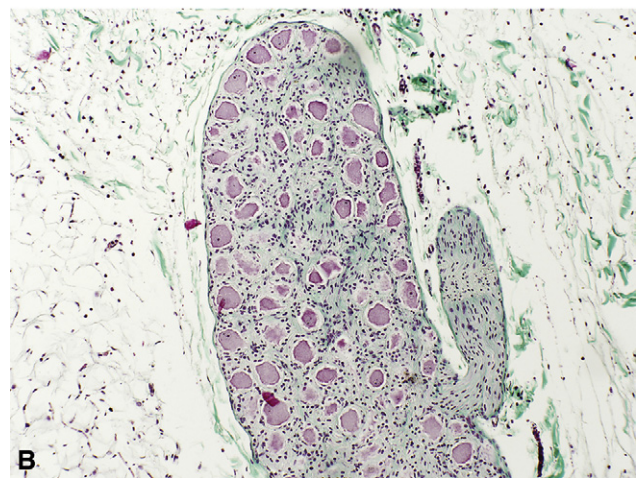
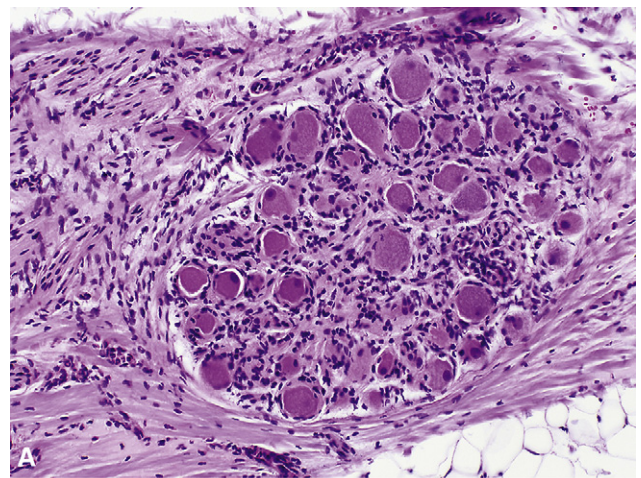


FIGURE E3. A, Irreversibly injured nerve bundle identified within the high-intensity focused ultrasound beam caused by the Epicor UltraCinch LP (hematoxylin and eosin stain). B, Ganglionic plexi ablated by the Epicor UltraWand LP (Masson trichrome stain).

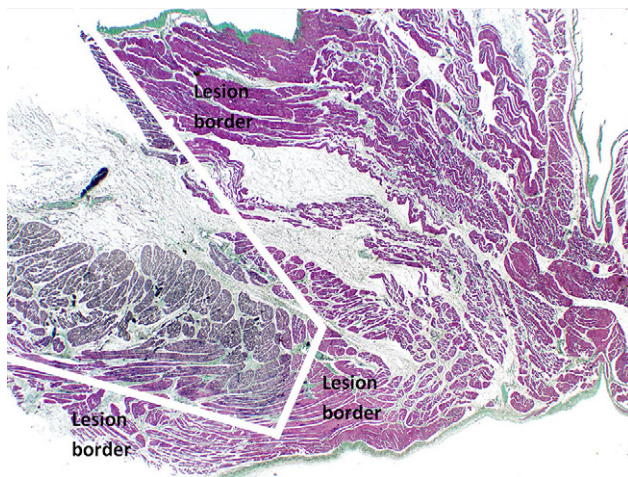


FIGURE E2. Oblique lesion presumably related to the shape of the target tissue and the orientation of the high-intensity focused ultrasound lesion.

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