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## Reduction in left atrial and pulmonary vein dimensions after ablation therapy is mediated by scar

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28 **Structured abstract**

29 Background: Ablative pulmonary vein isolation (PVI) decreases pulmonary vein (PV) and left atrial  
30 (LA) dimensions in atrial fibrillation (AF) patients. These changes are attributed to reverse structural  
31 remodeling following sinus rhythm restoration but evidence is lacking. We hypothesized that the  
32 downsizing is directly caused by the ablative energy and subsequent scar formation.

33 Methods: We studied cardiac magnetic resonance imaging in 21 paroxysmal AF patients before and 3  
34 months after successful PVI and in healthy sheep (n=12) before and after PVI of the right PV only.

35 Results: PVI decreased the PV diameter in patients and sheep by 11.0(10.3) and 9.2(11.0)%, ( $p < 0.001$   
36 and  $p = 0.020$ ), respectively. The control left PVs in sheep were unchanged. A linear correlation existed  
37 between the extent of PV scar and PVI-induced decrease in PV diameter in patients.

38 After PVI, the LA volume decreased (103(38) vs. 92(31)ml, pre- vs. post-ablation, respectively,  
39  $p = 0.006$ ), while the right atrial (RA) volume was unchanged in patients. A decrease in active emptying  
40 fraction after ablation (26.5(10.7) vs. 21.8(10.6)%, pre- vs. post-ablation,  $p = 0.031$ ) was associated  
41 with reduced contractility of the PV walls ( $p = 0.004$ ). The contractility of the LA walls was unaltered  
42 ( $p = 0.749$ ).

43 Conclusion: The ablation-induced PV diameter reduction was similar in patients with AF and healthy  
44 sheep without AF and was associated with PV scar extent. The volume only decreased in LA and not  
45 RA after PVI, and wall contractility decreased only in ablated sites. Therefore, the PVI-induced atrial  
46 downsizing is caused by the ablative energy and subsequent scar formation.

47

48 Key Words

49 Atrial fibrillation, Ablation scar, Pulmonary vein isolation, Cardiac magnetic resonance,  
50 Atrial contractility.

51

52

## 53 **1. Introduction**

54 Atrial fibrillation (AF) initiates a structural remodeling of the atrial myocardium involving  
55 loss of contractile fibers and an increase in interstitial collagen accumulation (fibrosis).(1, 2) Often,  
56 AF patients have enlarged atria and a reduced atrial contractile function.(3) Ablative pulmonary vein  
57 isolation (PVI) is a therapeutic option for AF when pharmacological therapy fails and is recommended  
58 in drug-resistant patients with paroxysmal AF (episodes of AF lasting less than 1 week).(4) On long-  
59 term follow-up, PVI cures 59% of paroxysmal AF patients.(5)

60 Successful PVI ablation causes a decrease in PV and left atrial (LA) dimensions within  
61 months after the ablation procedure.(6-8) This reduction in PV and LA dimensions is attributed to a  
62 decrease in AF burden and thereby reverse structural remodeling in the atria.(9-12) Reverse  
63 remodeling is a return towards the pre-disease state and involves the decrease of structural  
64 (hypertrophy, fibrosis, and atrial dilatation) and functional (decreased contractility) abnormalities.(13)  
65 However, there is no evidence underlying the association between the PVI-induced reduction in atrial  
66 dimensions and reverse remodeling on the one hand, and the subsequent scar formation on the other.

67 We hypothesized that the effect of ablative energy and subsequent scar formation explain the  
68 reduction in PV and LA dimensions. With cardiac magnetic resonance (CMR), we therefore studied  
69 PVI-induced changes in 1) PV dimensions, atrial volumes and LA contractility in AF patients with  
70 successful ablation and 2) PV dimensions in healthy sheep without AF. We anticipated that reverse  
71 structural remodeling initiated by normalization of the cardiac rhythm 1) only occurs in the setting of  
72 AF remodeling, 2) similarly takes place in the LA and right atrium (RA), 3) is characterized by an  
73 increase in contractile fibers and thereby contractility, and 4) exert an effect on LA dimensions in all  
74 cardiac phases. We tested this in patients with paroxysmal AF that were successfully treated with PVI  
75 ablation and in sheep without AF, and provide evidence that the changes in LA and PV diameter are  
76 the result of the ablative energy delivery and subsequent scar formation and not of reverse remodeling  
77 induced by decreased AF-burden.

## 78 **2. Methods**

79

### 80 2.1 Patient selection

81 Fifty-one paroxysmal AF patients referred for a first PVI ablation included in a previously  
82 published study (14) were retrospectively analyzed. The study was approved by the Institutional Ethics  
83 Committee (Comité de Protection des Personnes Sud-Ouest et Outre Mer III, approval reference 2012-  
84 A01494-39). Informed consent was obtained from all patients. All patients were re-catheterized 3  
85 months after the ablation procedure to test for recurrence of electrical PV-LA conduction. We included  
86 only patients with a clinically successful PVI defined as presence of sinus rhythm during the post-  
87 ablation CMR and re-catheterization both scheduled 3 months after the ablation procedure. Additional  
88 exclusion criteria were comorbidity of valvular disease, heart failure, administration of ablative  
89 radiofrequency (RF) outside the PVs, AF during pre-ablation CMR. Twenty-one paroxysmal AF  
90 patients were included of whom 7 were female (33%). Their age was 62(9) years and body mass index  
91 was 26.6(3.5) kg/m<sup>2</sup>. AF diagnosis had been made 60[60] months previously.

92

### 93 2.2 Patient CMR acquisition

94 CMR studies were conducted on a 1.5 Tesla system (MAGNETOM AVANTO, Siemens,  
95 Erlangen, Germany) equipped with a 32-channel body coil and a 18-channel cardiac coil. Cine  
96 imaging was performed by ECG-gated steady-state free precession pulse sequence during breath-  
97 holding in order to acquire a stack of 4-chamber images covering the entire heart (slice thickness 6mm  
98 without interslice gap) as well as a 2-chamber slice (slice thickness 6mm; temporal resolution: 15ms).

99 A late gadolinium enhancement (LGE) CMR acquisition was begun 17 minutes after  
100 intravenous injection of 0.2mmol/kg gadoterate meglumine (Guerbet, Aulnay-sous-bois, France) and  
101 consisted of a trans-axial three-dimensional orientation using an ECG-gated and respiration-navigated  
102 gradient-echo pulse sequence.

103 The following imaging parameters were used: in-plane resolution = 1.25 x 1.25mm<sup>2</sup>; flip angle  
104 = 22°; echo time = 2.4 ms; repetition time = 6.1 ms; generalized auto-calibrating partial parallel

105 acquisition (GRAPPA), acceleration factor of 2 with 42 reference lines. The pre-ablation CMR was  
106 performed within 3 days before the ablation procedure, while the post-ablation CMR was performed  
107 91±29 days after the pre-ablation CMR.

108

### 109 2.3 Patient ablation procedure

110 The ablation procedure has previously been described in depth.(14) Shortly, patients were  
111 randomized to complete PVI ablation with either a conventional point catheter (NAVISTAR  
112 THERMOCOOL, Biosense Webster, Irvine, USA; n=11/21) or a circular catheter (nMARQ, Biosense  
113 Webster; n=10/21). Endocardial LA access was achieved by trans-septal puncture and followed by  
114 heparin administration (0.5-0.8mg/kg). RF energy was delivered for 60s per application in a unipolar  
115 mode with a temperature limitation of 45°C and simultaneous irrigation with 0.9% saline (60mL/min).  
116 All patients had successful immediate procedural PVI confirmed by activation mapping that persisted  
117 after adenosine administration. We consider that the variations in lesion localization within catheter  
118 type differ equally when comparing both types.

119

### 120 2.4 Sheep CMR acquisition

121 The sheep study was carried out in accordance with the EU Directive 2010/63/EU for  
122 protection of animals used for scientific purposes and approved by the local ethical authorities at  
123 University of Bordeaux, France (approval number 7995).

124 CMR acquisition was performed *in-vivo* in healthy female sheep (n=12, 2-3 years old, 54(4)  
125 kg; sheep strain: Charmoise) before and 3-4 months after RF ablation of the right pulmonary vein  
126 (RPV). The sheep were anesthetized (pre-medication: 20 mg/kg ketamine + 0.1 mg/kg acepromazine,  
127 induction: 1 mg/kg propofol, maintenance: 2 % isoflurane) before placed on their back on the scanner  
128 table. The CMR studies were conducted on a 1.5 Tesla system (MAGNETOM Aera, Siemens,  
129 Erlangen, Germany) equipped with a 32-channel body coil and an 18-channel cardiac coil. Cine  
130 imaging was performed by ECG-gated steady-state free precession pulse sequence during forced  
131 breath-hold in order to acquire a stack of trans-axial images. Slice thickness was 4 mm (n=7) or 6 mm  
132 (n=5) and did not differ between pre- and post-ablation CMR in the same animal.

133 The following acquisition parameters were used: in-plane resolution = 1.3 x 1.3 mm<sup>2</sup>; flip  
134 angle = 58°; bandwidth = 992 Hz/pixel; echo time = 1.34 ms; repetition time = 21.98 ms; GRAPPA;  
135 acceleration factor of 3 with 75% partial Fourier acquisition. The pre-ablation CMR was performed  
136 within 1 week before ablation, while the post-ablation CMR was performed 120±11 days after the pre-  
137 ablation CMR.

138

### 139 2.5 Sheep ablation procedure

140 The sheep were catheterized via the femoral veins under general anesthesia and sterile  
141 conditions. LA access was achieved by trans-septal puncture using a steerable sheath. A circular  
142 ablation catheter (PVAC Gold, Medtronic, Minneapolis, USA) was placed in the RPV ostium under  
143 fluoroscopy guidance, and RF energy was administered 2 x 60s with bipolar:unipolar 2:1 phasing and  
144 a temperature limitation of 55 °C. The ablation reference electrode was placed on the lower back of  
145 the sheep. The animals recovered under surveillance for 1 week before returning to a hosting farm.

146

### 147 2.6 Image analysis

148 Only pre- and post-ablation images containing the same anatomical landmarks (the sternum,  
149 the vertebrae, and the aorta) in similar stack slices and being without artefacts in the LA and PV  
150 regions were included in the analysis. The PV diameter was measured at the ostium at the moment of  
151 maximum LA dilatation (just before opening of the mitral valve) in the patients (figure 1A) and sheep  
152 (supplementary figure 1) if the PVs remained temporally in plane in both pre- and post-ablation  
153 images.

154 In the patients, we analyzed the LA volume at the moment of maximum LA dilatation, and  
155 immediately before (pre-contraction) and after (minimum LA volume) atrial contraction by tracing the  
156 endocardial LA contours on each slice of the 4-chamber stack while excluding the PVs and LA  
157 appendage (LAA) in the software Syngo.via (Siemens, Erlangen, Germany; figure 1C). The volume  
158 was calculated as the sum of each LA area multiplied by the slice thickness. The maximum RA  
159 volume was similarly measured while excluding the caval veins from the RA areas (figure 1C).

160 We defined the total LA ejection fraction as (maximum LA volume – minimum LA  
161 volume)/maximum LA volume\*100%, the LA expansion index as (maximum LA volume – minimum  
162 LA volume)/minimum LA volume\*100%, the LA conduit fraction as (maximum LA volume – pre-  
163 contraction LA volume)/maximum LA volume\*100%, and the LA active emptying fraction as (pre-  
164 contraction LA volume – minimum LA volume)/pre-contraction LA volume\*100%.

165 We analyzed local LA strain and motion fraction throughout the cardiac cycle with a feature-  
166 tracking algorithm previously described.(15, 16) LA endocardial markers (>30) were positioned on a  
167 single phase of the cardiac cycle, and longitudinal strain (tangential deformation to the considered LA  
168 wall) and radial motion fraction (towards the LA center of mass) of defined regions were computed.  
169 We identified the anterior and posterior wall of the right superior PV (RSPV) and right inferior PV  
170 (RIPV) as well as the posterior wall of the left superior PV (LSPV; figure 1B). The tracking of the  
171 slightly kinked anterior LSPV wall towards the LAA was less precise and therefore excluded from the  
172 analysis. Also, 3 LA regions outside the PVs were defined: a septal LA wall in the 4-chamber stack (in  
173 the slice with the RSPV; figure 1B) and a posterior and an inferior LA wall in the 2-chamber slice  
174 (figure 1E). The PV ostia and LAA were excluded from these regions. The temporal beginning of the  
175 strain/motion fraction curve was the moment immediately after atrial contraction, and we considered  
176 the maximum magnitude of the last 20% of the curve as an indicative of local contractility. A  
177 contractility index of the PV regions and of the LA regions was calculated in each patient as the sum  
178 of the logarithmically transformed values of local contractility (2 values per wall region: 1 value each  
179 from the strain and the motion fraction curve. The PV contractility index was calculated based on the 5  
180 PV regions; the LA contractility index on the 3 LA regions).

181 Scar burden was quantified on the LGE CMR images (figure 1G) using the MUSIC software  
182 (IHU Liryc, Bordeaux, France) as previously described.(14) Shortly, the LA wall was manually traced,  
183 and LGE was segmented using histogram analysis, applying the full width at half maximum method.  
184 Thresholding of myocardial voxel intensity (50-70% of maximum signal intensity) was performed,  
185 and scar in the PVs and LA was quantified as the difference between pre- and post-ablation  
186 measurements and expressed in ml.



187

## 188 2.7 Histology

189 Three sheep hearts were explanted after post-ablation CMR acquisition in accordance with the  
190 ethically approved euthanasic methods. The RPV and left PVs (LPV) were dissected and fixated in  
191 paraformaldehyde (4%) at 5°C for 2 weeks before cut in the longitudinal direction thus including the  
192 atrial-PV junction and the distal PV. Specimen were dehydrated automatically (Leica HistoCore Pearl  
193 processor, Wetzlar, Germany) before being embedded in paraffin and sectioned with a microtome  
194 (6µm slice thickness). A Masson's trichrome staining was applied to the slices to visualize  
195 cardiomyocytes (red), nuclei (black), and collagen (green) before digitally scanned with an objective x  
196 20.

197

## 198 2.8 Statistical analysis

199 Data are expressed as mean (standard deviation) or median [interquartile range] depending on  
200 normality tested with a Shapiro Wilk's test. Pre- and post-ablation CMR data were tested with a two-  
201 tailed paired Student's t-test or a Wilcoxon signed rank test, as appropriate. P-values<0.05 were  
202 considered as statistically significant. The 95% confidence intervals (CI<sub>95%</sub>) are included for  
203 parametric testing.

204 A mean of ablation-induced change in PV diameters (post-ablation diameter – pre-ablation  
205 diameter / pre-ablation diameter \*100%) was calculated in each patient and was tested against a  
206 theoretical mean of 0 with a two-tailed one-sample Student's t-test. The change in diameter after  
207 ablation of the ablated RPV and a mean change in diameter of the non-ablated LPVs were calculated  
208 in each sheep before similar testing and Bonferroni correction (0.05/2). A two-tailed unpaired  
209 Student's t-test was used to test for differences in ablated PVs between patients and sheep.

210 A linear regression model was fitted to the mean PV diameter change in patients as a function  
211 of the logarithmically transformed extent of PV ablation scar. A mixed effect linear model was applied  
212 to the PV and LA contractility indexes.

213

### 214 3. Results

215 Not all PVs were accessible for analysis. The left inferior PV (LIPV) in the AF patients was  
216 rarely visible and comparable between the pre- and post-ablation datasets (n=4) and was discarded  
217 from the analysis. In patients, 14 RIPVs, 18 RSPVs, and 16 LSPVs were analyzed. None had left or  
218 right single ostium PV or unilateral triple PVs. In sheep, 11 RPVs, 10 LSPVs, and 11 LIPVs were  
219 measured (a RIPV is absent in sheep).

220

#### 221 3.1 Ablation-caused decrease in PV diameter

222 Patients: PVI ablation caused a decrease in PV diameter in the AF patients (18.9(4.2) vs.  
223 16.5(3.8) mm, pre- vs. post-ablation, respectively; PVI-induced change: -11.0 (10.3) %, CI<sub>95%</sub>=[-15.9 -  
224 6.0], p<0.001, hypothesis test against theoretical mean equaled 0; figure 2A)

225 Sheep: The PV diameter of the ablated RPV in sheep also decreased after ablation (13.8(2.3)  
226 vs. 12.3(1.6)mm, pre- vs. post-ablation, respectively; PVI-induced change: -9.2(11.0)%, CI<sub>95%</sub>=[-16.6 -  
227 1.8], p=0.020; figure 2B), whereas the control left PVs remained unchanged (15.6(2.3) vs.  
228 15.5(2.2)mm, pre- vs. post-ablation, respectively; PVI-induced change: 1.5(12.0)%, CI<sub>95%</sub>=[-6.6 9.5],  
229 p=0.689; figure 2B).

230 The percentage decrease in ablated PV diameter was similar (-11.0(10.3) and -9.2(11.0)% in  
231 patients and sheep, respectively, CI<sub>95%</sub>=[-6.4 10.0], p=0.660).

232

#### 233 3.2 PV scar

234 Patients: Three months after PVI, ablation scar was observed in the PV regions in all patients  
235 (7.4 (2.1)ml; figure 1H). LA scar was observed in 1 patient (1.5ml). A negative linear relation existed  
236 between ablation-induced PV scar and PV diameter change (R<sup>2</sup>=0.388, H<sub>0</sub> slope equal to 0: p=0.004,  
237 slope = -44.3 CI<sub>95%</sub>=[-72.8 -15.9]; figure 2C).

238 Sheep: Three months following PVI, acellular collagenous ablation scar was observed in the  
239 RPVs of sheep (figure 2D left panel), but not in the LPVs (figure 2D right panel).

240

241 3.3 LA volume reduction in AF patients

242 The volume analysis was based on 20 AF patients because the CMR stack did not cover the  
243 whole atrium in 1 patient. The maximum and pre-contraction LA volume in patients decreased by  
244 9(18) and 9(15) %, respectively, after ablation, whereas the minimum LA volume remained  
245 unchanged (table 1). The maximum RA volume did not change after successful PVI in patients  
246 (81(23) vs. 78(27) ml, pre- vs. post-ablation,  $CI_{95\%}=[-8.2\ 13.2]$ ,  $p=0.629$ ).

247

248 3.4 Decrease in active LA emptying fraction after successful PVI in AF patients

249 The active LA emptying fraction decreased by 19[42] % after ablation in the patients (table 1).  
250 The total LA ejection fraction remained stable after ablation as reflected in an unaltered passive LA  
251 conduit fraction (table 1). We observed a tendency towards a lower LA expansion index after ablation  
252 (table 1). Heart rate did not change in the patients (62[13] vs. 66[20] bpm; pre- vs. post-ablation,  $p=$   
253 0.170) and, therefore, did not constitute a confounding factor.

254

255 3.4 Decrease in PV wall contractility in AF patients

256 Figure 3 shows the longitudinal strain curves before (A) and after (B) ablation of the RSPV  
257 posterior wall in patients. The maximum magnitude of the last 20% of the strain curve was considered  
258 an indicative of local contractility. The PV contractility index decreased after ablation (5.57 (2.43) vs.  
259 4.69 (2.38) arb. unit, pre- vs. post-ablation,  $CI_{95\%}=[0.26\ 1.50]$ ,  $p=0.004$ ), while the LA contractility  
260 index was unaltered by PVI (5.74 (2.17) vs. 5.51 (2.18) arb. unit, pre- vs. post-ablation,  $CI_{95\%}=[-0.37$   
261 0.82],  $p=0.749$ ; figure 3C).

262

263 Thus, in patients with AF, we observed that the LA volume reduction after PVI ablation was  
264 associated with a local decrease in contractility in the PV regions in which RF energy was delivered,  
265 but not in regions remote from the site of energy delivery.

266

## 267 **4. Discussion**

268 We investigated the changes in atrial dimensions and mechanics in paroxysmal AF patients  
269 after successful PVI and in healthy sheep after ablation in a single PV. Our results showed that the  
270 diameter of ablated PVs decreased similarly in the patients and in healthy sheep, and that a linear  
271 correlation existed between the decrease in PV dimension and extent of PV scar in patients. The  
272 maximum volume of the LA was smaller, whereas that of the RA remained unchanged, after PVI in  
273 patients. Finally, the PV walls contracted less, while the contractility of the LA walls remote from RF  
274 energy delivery did not alter after PVI in patients.

275

### 276 4.1 Reverse structural remodeling

277 AF causes structural remodeling of the atrial tissue by fibrosis, fat accumulation, myocardial  
278 hypertrophy, and loss of contractile fibers.(1, 2, 17) A consequence of structural remodeling is atrial  
279 dilatation that is common in AF patients and is an independent risk factor for AF in humans.(3, 18)  
280 Observations of counteracting processes in the atria have been made upon restoration of sinus rhythm,  
281 and this phenomenon has been termed reverse remodeling.(13)

282

### 283 4.2 PVI-induced decrease in PV dimensions

284 A PVI-induced reduction in PV diameter of about 10% is observed by others.(7, 10) AF  
285 patients have enlarged PVs compared to control patients, and structural remodeling due to AF burden  
286 is considered to be the cause.(19) Therefore, a reverse structural remodeling by cessation of AF has  
287 been proposed as causing the decrease in PV dimensions after successful PVI.(10) We observed that  
288 PVI ablation in healthy sheep without AF, in which structural remodeling due to arrhythmia cannot be  
289 present, caused a similar chronic decrease in PV diameter as in AF patients. It is therefore more likely  
290 that the PV size reduction is the direct causal effect of ablative energy delivery and subsequent scar  
291 formation rather than reverse remodeling processes. Indeed, we observe a statistically significant  
292 linear correlation between scar extent in the PVs and PV diameter decrease after successful PVI in AF  
293 patients.

294 Healthy animals served as a control to the successfully ablated AF patients instead of patients  
295 with AF recurrence because in the case of AF recurrence even a persistently isolated PV (as observed  
296 on re-do catheterization) is still subject to AF remodeling (and thereby continuous dilatation). Indeed,  
297 dilatation of RSPV is observed in patients still in AF after PVI.(7).

298

#### 299 4.3 Ablation-induced reduction in LA volume

300 A meta-analysis of 8 studies supports our findings of a decrease in LA volume after successful  
301 AF ablation,(8) and the LA volume decrease itself has been interpreted as a marker of reverse  
302 remodeling.(9, 11, 12) Ausma and colleagues report that 2 months of sinus rhythm (after 4 months of  
303 AF) decreased AF-induced atrial myolysis in goats, although a complete normalization was (still) not  
304 observed after 4 months of sinus rhythm.(13) This emphasizes that reverse remodeling processes does  
305 occur, but at a slow rate and most likely beyond the 3-month blinding period after AF ablation.

306 In patients with AF recurrence, in whom a reverse structural remodeling is not expected, a  
307 similar decrease in LA volume has been observed after AF ablation.(10) Thus, the chronic effects of  
308 ablative energy must directly have caused the LA volume reduction seen after PVI, rather than the  
309 restoration of sinus rhythm. Indeed, the volume of the RA, in which no RF energy was delivered,  
310 remained unchanged 3 months after successful PVI in our patients, although AF was equally absent as  
311 in the LA.

312

#### 313 4.4 Loss of contractility at ablated sites

314 Our observation of a decrease in active LA emptying fraction after PVI is confirmed by  
315 others.(8) We reason that a reverse remodeling that includes normalization of the contractile fibers,  
316 should lead to an increase and not a decrease in the active LA function. An increase in active LA  
317 emptying fraction from 22 to 33% is observed after successful PVI by Marsan et al.(20) However,  
318 45% of these patients were medicated with angiotensin receptor blocker that is known to attenuate AF-  
319 induced atrial fibrosis.(21)

320 We report that the contractility of 3 LA regions remote from the sites of RF delivery was  
321 unchanged after PVI. This observation questions a global reverse remodeling based on restoration of

322 sinus rhythm. Instead, we observed a decrease in contractility in the PV walls, possibly due to loss of  
323 myocytes caused by the ablative energy. In alignment with our findings, others report a positive  
324 correlation between the amount of ablation scar (measured by late gadolinium enhancement) and a  
325 decrease in total LA ejection fraction.(22)

326

#### 327 4.5 Stretch-reduction as an antiarrhythmic factor

328 A reduction in PV and LA dimensions, under the assumption of unaltered LA pressure,  
329 decreases wall stress according to Laplace's law. Because increased atrial and PV stretch is  
330 proarrhythmic,(23, 24) and relief of chronic stretch is antiarrhythmic,(25) the ablation-induced  
331 decrease in atrial dimensions may play a role in preventing AF by reduction in local wall stress.  
332 Indeed, the plasma concentration of B-type natriuretic peptide (BNP), a marker of atrial stretch, is  
333 decreased after successful PVI.(11) The authors observed a correlation between post-ablation  
334 arrhythmia burden and BNP, and they proposed BNP as a marker of reverse structural remodeling.  
335 However, it is more likely that the ablation scar decreases atrial stretch by dimension reduction and  
336 thereby correlates with a lower arrhythmia burden.

337 Our observation of an unaltered minimum LA volume, despite smaller maximum LA volume,  
338 after ablation indicates that PVI prevented LA dilatation by a stiffening of the chamber. Indeed, we  
339 observed a tendency towards a lower expansion index after PVI. A future goal of research is to  
340 optimize ablation therapy such that local ablative energy delivery is sufficient to decrease LA and PV  
341 dimensions to prevent proarrhythmic dilatation but small enough to prevent loss of contractile  
342 function.

343

#### 344 4.6 Study limitations

345 The reproducibility of CMR before and after PVI prevented us from analyzing all PVs. Also,  
346 the in-plane resolution was  $1.25 \times 1.25 \text{mm}^2$  in patients, and therefore the measurement of radial strain  
347 of the LA myocardial wall (changes in myocardial thickness) during the cardiac cycle was not  
348 feasible. We instead analyzed the motion fraction of the wall towards the LA center of mass.

349

350 4.7 Conclusion

351 We demonstrated that the reduction in LA and PV dimensions after successful PVI is  
352 attributed to the direct effect of ablative energy delivery and scar formation rather than reverse  
353 structural remodeling based on restoration of sinus rhythm. The curative potential of reverse structural  
354 AF remodeling is therefore smaller than previously estimated. An antiarrhythmic effect of PVI may lie  
355 in the downsizing of the LA and PVs.

356

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361

362 **6. Discloses**

363 The authors have no disclosures.

364

365 **7. Author statements**

366 LAG: Conceptualization, Methodology, Software, Validation, Formal analysis,  
367 Investigation, Data Curation, and Writing (original draft). NAJ: Formal analysis, Investigation, Data  
368 Curation, and Writing (review & editing). DEH: Methodology, Writing (review & editing), and  
369 Investigation. JN: Methodology and Investigation. JL: Methodology, Data Curation, Software, and  
370 Writing (review & editing). NK: Methodology, Data Curation, Software, and Writing (review &  
371 editing). BQ: Project administration and Resources. HC: Conceptualization, Supervision, and  
372 Resources. RC: Conceptualization, Writing (original draft), Funding acquisition, Validation, and  
373 Supervision. LRCD: Conceptualization, Writing (original draft), Funding acquisition, Validation, and  
374 Supervision.

375

376 **8. Data statement**

377           The data supporting the study findings can upon reasonable request be made available from  
378 the corresponding author.

379

380



381 **9. References**

382

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- 462

463 **10. Tables**

464 Table 1: Left atrial dimensions and mechanics before and after ablation in patients

465

	Pre-ablation	Post-ablation	Pre-ablation vs. post-ablation
Maximum LA volume, ml	103.4(37.6)	91.6(31.1)	CI <sub>95%</sub> =[3.9 19.8]; p=0.006
Pre-contraction LA volume, ml	87.9(31.3)	78.8(26.5)	CI <sub>95%</sub> =[3.3 14.9]; p=0.004
Minimum LA volume, ml	68.2[47.0]	57.9[33.6]	p=0.391
Total LA ejection fraction, %	37.3(9.7)	32.5(10.3)	CI <sub>95%</sub> =[-0.1 9.6]; p=0.053
Active LA emptying fraction, %	26.5(10.7)	21.8(10.6)	CI <sub>95%</sub> =[0.5 9.1]; p=0.031
Passive LA conduit fraction, %	14.5(5.9)	13.7(6.4)	CI <sub>95%</sub> =[-3.7 5.3]; p=0.701
LA expansion index, %	63.0(24.7)	51.6(23.9)	CI <sub>95%</sub> =[-0.1 22.9]; p=0.051

466

467 Legend table 1: The LA volumes were manually measured on CMR images at the moment of  
 468 maximum LA dilatation, and immediately before and after atrial contraction. The mechanical  
 469 parameters were calculated from these volumes. Values are expressed as mean(standard deviation) or  
 470 median[interquartile range] dependent on normality. The 95% confidence intervals are included if  
 471 testing was parametric.

472

473 **11. Figure legends**

474

475 Figure 1: CMR images in AF patients

476 Four-chamber slice with RSPV and LSPV at the moment of maximum LA dilatation in patients before  
477 (A) and after (B) PVI ablation. Dotted lines: PV diameters. Closed circles: LA septal wall. Open  
478 circles: RSPV anterior wall. Closed triangle: RSPV posterior wall. Open triangle: LSPV posterior  
479 wall. Four-chamber slice with RIPV before (C) and after (D) ablation. Dotted lines: RA and LA  
480 surface areas for volume estimation. Asterisks: RIPV anterior wall. Hashtag: RIPV posterior wall.  
481 Two-chamber slice before (E) and after (F) ablation. Open square: LA posterior wall. Closed square:  
482 LA inferior wall. LGE CMR before ablation (G) and after ablation (H). Note the scar formation in the  
483 PV walls after ablation.

484

485 Figure 2: Ablation-induced changes in PV diameter and scar

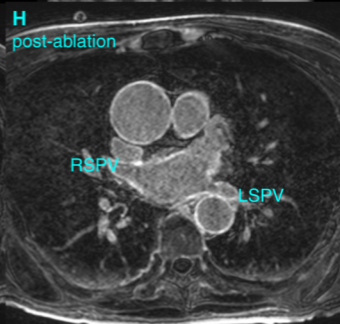
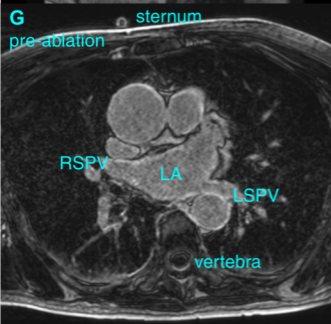
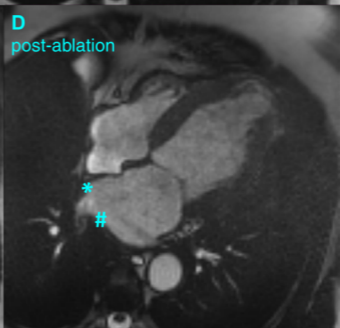
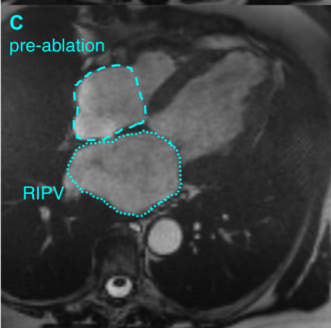
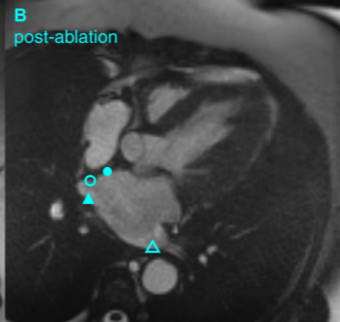
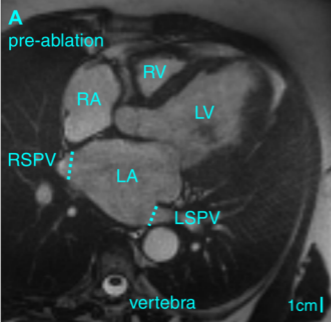
486 A: The PV diameter decreased after PVI. A mean of ablation-induced PV diameter change was  
487 calculated in each patient and hypothesis-tested against a theoretical mean of 0 (one sample Student's  
488 t-test). B: The diameter of the ablated RPV decreased after ablation in sheep, while the diameter of the  
489 control LPVs (intra-sheep mean) was unchanged. C: There existed a negative linear correlation  
490 between PV diameter change and extent of PV scar (logarithmically transformed).  
491 D: Collagenous ablation scar occurred in the RPV (left panel), but not in the LPV of sheep (right  
492 panel).

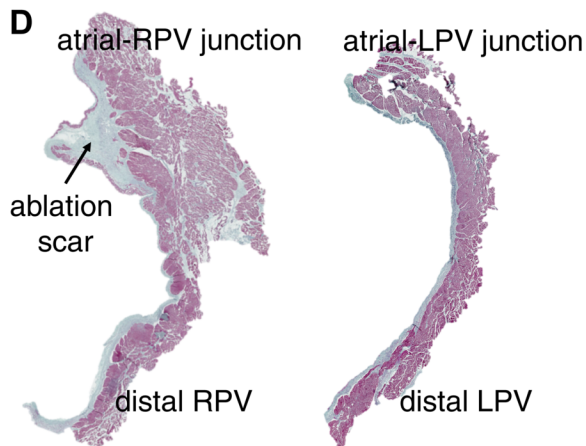
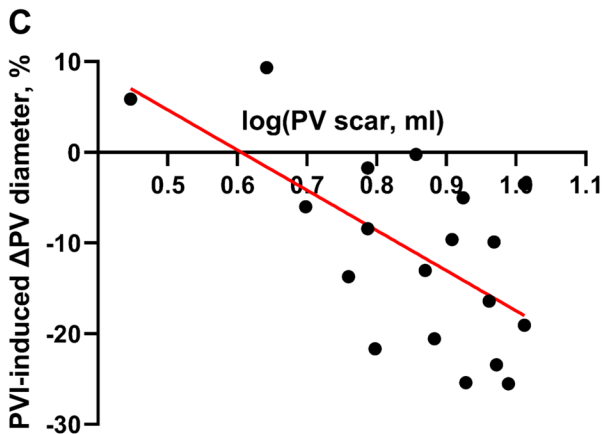
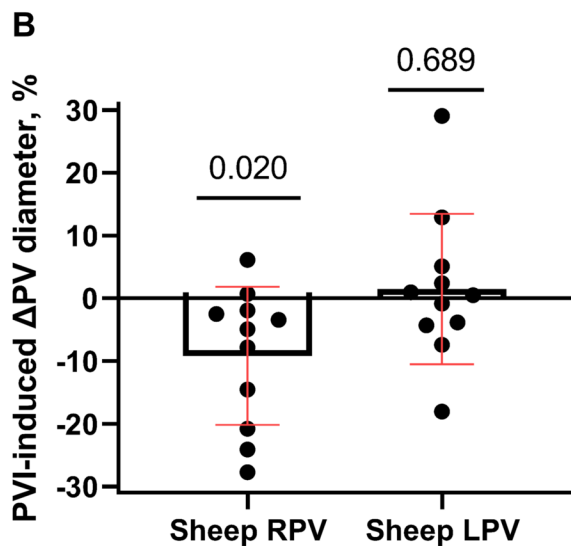
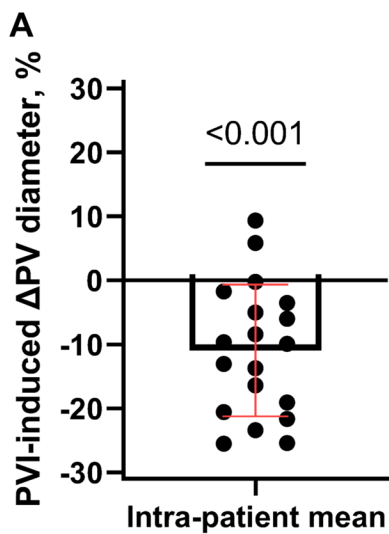
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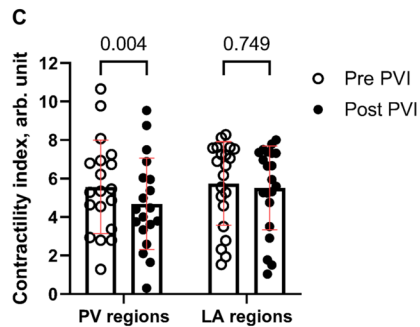
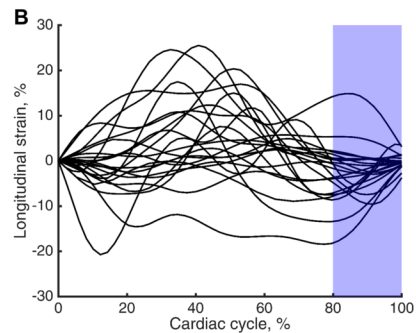
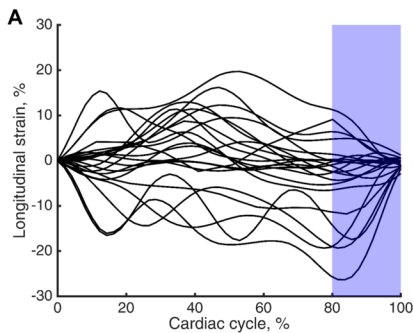
494 Figure 3: Regional wall contractility before and after ablation in patients

495 The longitudinal strain curves before (A) and after (B) ablation of the posterior RSPV wall where  $x=0$   
496 is the moment immediately after atrial contraction. We considered the last 20% of the curve as the  
497 atrial contractile phase (blue shade). Localized contractility was quantified as the magnitude of the  
498 strain during this phase. C: The PV contractility index decreased after ablation whereas the LA  
499 contractility index remained unchanged.

500





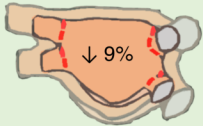


Successful PVI in AF patients causes:



LA volume

PV diameter

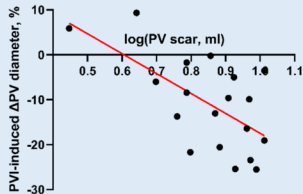


The LA and PV downsizing is caused by the **ablative energy** and subsequent **scar**:

1. Similar PV diameter reduction after PVI in healthy sheep without AF.



2. Correlation between extent of PV scar and PV diameter decrease after PVI in patients.



3. Unchanged RA volume after PVI in AF patients.



4. PVI-induced decrease in active LA emptying fraction in patients.



5. PVI-caused reduction in contractility in PV walls, but not in LA walls in patients.

