Rearrangement of Atrial Bundle Architecture and Consequent Changes in Anisotropy of Conduction Constitute the 3-Dimensional Substrate for Atrial Fibrillation

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Background—Anisotropy of conduction facilitates re-entry and is, therefore, a key determinant of the stability of atrial fibrillation (AF). Little is known about the effect of AF on atrial bundle architecture and consequent changes in anisotropy of conduction and maintenance of AF.

Methods and Results—Direct contact mapping was performed in left atria of goats with acute AF (n=6) or persistent AF (n=5). The degree and direction of anisotropic conduction were analyzed. Mapped tissue regions were imaged by high-resolution MRI for identification of endocardial and epicardial bundle directions. Correlation between endocardial and epicardial bundle directions and between bundle directions and anisotropic conduction was quantified. In persistent AF, epicardial bundles were oriented more perpendicularly to endocardial bundles than in acute AF (% angles <20° between epicardial and endocardial bundle directions were 7.63% and 21.25%, respectively; P<0.01). In acute AF, the direction of epicardially mapped anisotropic conduction correlated with endocardial but not with epicardial bundles. In persistent AF, the direction of anisotropic conduction correlated better with epicardial than with endocardial bundles (% angles <20° between direction of anisotropic conduction and bundle direction were 28.77% and 18.45%, respectively; P<0.01).

Conclusions—During AF, atrial bundle rearrangement manifests itself in more perpendicular orientation of epicardial to endocardial bundles. Propagation of fibrillation waves is dominated by endocardial bundles in acute AF and by epicardial bundles in persistent AF. Together with the loss of endo-epicardial electrical connections, rearrangement of atrial bundles underlies endo-epicardial dissociation of electrical activity and the development of a 3-dimensional AF substrate.

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Key Words: arrhythmia • atrial fibrillation • electrical dissociation • electrophysiology • epicardial mapping • remodeling

Mechanisms perpetuating atrial fibrillation (AF) are incompletely understood. Both in experimental and in clinical studies, persistent AF is sustained by multiple wavelets propagating throughout the atria,1,2 but also ectopic focal discharges and localized rotors have been proposed as a dominant activation pattern during AF.3–10

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In general, heterogeneity in electrophysiological properties promotes multiple wavelet re-entry.11,12 In particular, nonuniform anisotropy of conduction facilitates unidirectional block and can cause heterogeneity and local slowing of conduction.11,12 Both factors reduce the pathlength for re-entry and facilitate initiation and perpetuation of AF.13–17 At macroscopic scales, anisotropy of conduction velocity (CV) is surprisingly low both in right (RA)18–20 and in left atria (LA)21 (anisotropy ratio ranges from 1.2 to 3.2). However, at the microscopic scale, conduction is highly anisotropic.11 In atrial muscle bundles isolated from young individuals, an anisotropy ratio of =4.5 was reported, which even increased 2-fold when older patients were studied.13

Atrial architecture is highly complex. It not only shows 3-dimensional (3D) arrangements of circumferentially and longitudinally orientated muscle bundles but also sudden transitions in fiber architecture from the endocardial to the epicardial layer.22,23 Such transitions may promote conduction block and re-entry, especially in the presence of tissue fibrosis.24
The exact effects of the atrial bundle architecture on conduction anisotropy during AF and its changes caused by structural remodeling have not yet been determined. It is important to distinguish anisotropy of conduction velocity (which is based on CV in the various propagation directions) from anisotropy of conduction likelihood (ie, a parameter based on most frequently encountered propagation direction). The direction of the fastest conduction tends to correspond to local fiber orientation. However, the most frequently encountered propagation direction does not necessarily align with highest CV. In fact, anisotropy of conduction likelihood may reflect not only local fiber orientation but also the surrounding tissue architecture. Here, we have studied changes in endocardial and epicardial bundle orientation after 7 months of AF in goat LA and their relationship to the anisotropic behavior of fibrillation waves.

Methods
Open Chest Experiments and Tissue Harvesting
Sham-operated goats with acutely induced AF (aAF; n=6) and goats with AF persisting for 7 months (persAF; n=5) were studied (see online-only Data Supplement for details). After electrical cardioversion, atrial effective refractory periods were determined. A square contact mapping array (256 channels; interelectrode distance, 1.5 mm; Figure IA in the online-only Data Supplement) was positioned on the LA wall, and conduction during epicardial pacing outside the mapping area near the 4 corners was recorded. AF was re-induced, and fibrillation electrogram recordings (4 s) were analyzed using a wave mapping algorithm (see online-only Data Supplement) described previously. Anisotropic behavior is defined as the property of being direction dependent. To study anisotropic conduction of fibrillation waves, 2 types of anisotropy were distinguished.

First, we determined anisotropy of CV (ie, fastest versus slowest propagation direction). This was characterized by degree of anisotropy of CV (ie, the ratio between the long axis and the short axis of the ellipse fitted through local conduction vectors) and direction of anisotropy of CV (ie, the direction of the fastest propagation). Second, we determined anisotropy of conduction likelihood (ie, the degree of spread of propagation directions) and the direction of anisotropy of conduction likelihood (ie, the most frequently observed propagation direction). See online-only Data Supplement for a detailed description of anisotropy calculation.

Analysis of MRI Images
Original high-resolution 3D MRI data sets (acquisition details and Movie I in the online-only Data Supplement) were reconstructed to yield a stack of 2-dimensional (2D) images in a plane parallel to the epicardial recording surface (Figure 1B) and were used to determine the direction of endocardial bundles and epicardial fibers (Figure 1C and 1D). A 16×16 grid, spaced to contain 1 recording electrode each in the center of any grid square, was overlaid on these 2D reconstructions. For each electrode position, the orientation of the endocardial and epicardial bundles within that square was determined. Squares not containing tissue were excluded from analysis. Only MRI images located within <220 µm of the epicardial boundary were used for epicardial bundle identification.

Quantification of Anisotropy
Anisotropy of Conduction During AF
Epicardial unipolar fibrillation electrogram recordings (4 s) were analyzed using a wave mapping algorithm (see online-only Data Supplement) described previously. Anisotropic behavior is defined as the property of being direction dependent. To study anisotropic conduction of fibrillation waves, 2 types of anisotropy were distinguished.

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Anisotropy of Conduction During Pacing
Pacing (200-ms cycle length) was performed outside each of the 4 corners of the mapping array. As a result of hemodynamic instability, pacing was not possible in 1 persAF goat. For each pacing site (n=4), 4 consecutive beats were analyzed. All analyzed beats (n=16) were merged per goat, and the degree and direction of anisotropy of CV were determined by ellipse fitting. Because the pacing location dictated the conduction direction, the degree of anisotropy of conduction likelihood was not calculated.
Figure 2. Atrial refractory period measurements (AERP) at different pacing cycle lengths for acute atrial fibrillation (aAF) and persistent AF (persAF). \( ^{*} P<0.05 \) between aAF and persAF.

**Heterogeneity of Conduction Anisotropy and Bundle Orientation**

To quantify and compare the organization of the 2 types of anisotropy, a heterogeneity index (HI) was calculated. For each electrode, the mean absolute angle difference between the direction of anisotropy of CV or conduction likelihood at that electrode and the direction of anisotropy of CV or anisotropy of conduction likelihood at its 8 surrounding electrodes was determined. The HI was calculated as the mean of all absolute angle differences. The same HI calculation was used to quantify the organization of epicardial and endocardial bundle orientation.

**Directional Coherence Between Electrophysiological and Structural Data**

To assess the relationship between electrophysiological and structural data, directions of anisotropy of CV and anisotropy of conduction likelihood were compared with the directions of endocardial bundles and epicardial fibers, both in aAF and persAF. For quantitative analysis, angle differences were pooled within the aAF group and within the persAF group. Only absolute angle differences between 2 directions were used for analysis so that the minimal and maximal angle differences between the 2 compared directions equal 0° and 90°, respectively. For each comparison, the mean angle difference was calculated. As a metric, the ratio \( H_{\text{abs}} = \frac{\sum_{N} \text{small angle differences} (0°-20°)}{\sum_{N} \text{large angle differences} (90°-180°)} \) was calculated to quantify directional correlation.

**Statistical Analysis**

Data are expressed as mean±SD. Significance of differences in means between aAF and persAF was assessed using an unpaired Student \( t \) test. As angle differences were pooled for aAF and persAF, a mixed-effects ANOVA was performed to test for significance of differences in mean angle differences between these groups. For this purpose, a linear model was used with fixed effect of aAF or persAF and with random effect of the goat the angle difference belonged to. To test for the presence of a uniform distribution of angle differences between the 2 directions \( P_{\text{uniform}} \), a Kolmogorov–Smirnov test was used. There was no adjustment for multiple comparisons. \( P<0.05 \) were considered statistically significant.

### Table 1. Overview of AF Substrate Parameters

<table>
<thead>
<tr>
<th></th>
<th>AERP (ms)</th>
<th>Breakthroughs per s</th>
<th>Fibrillation waves per s</th>
<th>Width of Fibrillation Waves, mm</th>
<th>Conduction Velocity, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>aAF</strong> (n=6)</td>
<td>127±15</td>
<td>7.4±4.2</td>
<td>41.0±14.4</td>
<td>8.0±2.0</td>
<td>62.6±9.04</td>
</tr>
<tr>
<td><strong>persAF</strong> (n=5)</td>
<td>103±20*</td>
<td>32.7±10.0†</td>
<td>101.6±27.0†</td>
<td>5.9±0.9*</td>
<td>53.7±6.9*</td>
</tr>
</tbody>
</table>

*P<0.05; †P<0.001 aAF vs persAF.

**AF Substrate Complexity**

Atrial effective refractory periods (at cycle lengths >250 ms; Figure 2) and AF cycle length (Table 1) were significantly shorter in persAF than in aAF. Further electrophysiological parameters characterizing the AF substrate are listed in Table 1. As expected,4,5 the number of fibrillation waves and breakthroughs per second were higher in persAF compared with aAF, whereas the CV and width of fibrillation waves were lower. Figure 3 shows the representative wave maps and electrograms of aAF and persAF. In aAF (Figure 3A), a single AF cycle consists of only a few simultaneous waves, with no or few epicardial breakthroughs occurring. However, in persAF (Figure 3C), a single AF cycle consists of multiple simultaneous waves, with the occurrence of several breakthroughs. Furthermore, electrograms in persAF show more fractionation than in aAF. These results indicate the presence of a more complex AF substrate in the persAF group.

**Degree of Anisotropy of Conduction**

The degree of anisotropy of CV and anisotropy of conduction likelihood during pacing and AF is summarized in Table 1 in the online-only Data Supplement. During pacing, no significant differences were present in anisotropy of CV between aAF and persAF. Also during AF, anisotropy of CV was comparable in both groups. The degree of anisotropy of conduction likelihood, however, was significantly lower in persAF compared with aAF.

To study the relationship between the 2 aspects of anisotropic conduction, the angle differences between the fastest (direction of anisotropy of CV) and the most frequent direction (direction of anisotropy of conduction likelihood) are depicted in 2 rose diagrams (Figure IV in the online-only Data Supplement). In aAF and persAF, no clear correlation between the 2 directions was found (both \( R_{\text{s}} \) close to 0.5, ie, small and large angle differences were equal in frequency), but the distribution was more uniform in aAF than in persAF \( (P_{\text{uniform}}=0.22 \text{ versus } P_{\text{uniform}}=0.02) \). Mean angle differences between both anisotropic directions were not different between aAF and persAF (Table 2).

**Endocardial Versus Epicardial Bundle Orientation**

To study whether AF-related remodeling is accompanied by changes in atrial bundle orientation, the direction of endocardial bundles was compared with the direction of epicardial fibers in aAF and persAF (Figure 4). In aAF, endocardial bundle orientation often showed large angles to epicardial bundle orientation \( (R_{\text{s}}=0.44; P_{\text{uniform}}<0.001; \text{ Figure 4A}) \). However, in the persAF group, this was significantly more pronounced with an even larger fraction of large angles \( (R_{\text{s}}=0.19; P_{\text{uniform}}<0.001), \text{ Figure 4B} \).
P<0.05 versus aAF; Figure 4B), indicating that epicardial bundles were oriented more perpendicularly to endocardial bundles than in aAF (see Table 2 for mean angle differences and Results section in the online-only Data Supplement).

Table 2.  Mean Angle Differences for Comparison of Different Directions

<table>
<thead>
<tr>
<th></th>
<th>aAF (n=6)</th>
<th>persAF (n=5)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Directions of anisotropy of conduction velocity vs anisotropy of conduction likelihood</td>
<td>44.5±26.4°</td>
<td>47.3±27.0°</td>
<td>0.51</td>
</tr>
<tr>
<td>Directions of epicardial fibers vs endocardial bundles</td>
<td>47.6±26.8°</td>
<td>56.6±21.9°</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Relationship of Anisotropy to Tissue Substrate

All angle differences between the direction of anisotropy of CV and the direction of the endocardial and epicardial bundles are plotted in Figure 5A to 5D. In aAF, the direction of
anisotropy of CV corresponded well with endocardial bundle direction ($R_N=0.67$; $P_{\text{uniform}}<0.001$), whereas there was no correlation with the epicardial fiber direction ($R_N=0.54$; $P_{\text{uniform}}=0.08$). In contrast, in persAF, the direction of anisotropy of CV correlated better with epicardial bundle direction ($R_N=0.66$; $P_{\text{uniform}}<0.001$) and was oriented predominantly perpendicularly to the endocardial bundle direction ($R_N=0.34$; $P_{\text{uniform}}<0.01$).

The same analysis was performed for the direction of anisotropy of conduction likelihood (Figure 5E–5H). For aAF, the direction of anisotropy of conduction likelihood corresponded with the direction of the endocardial bundles ($R_N=0.64$; $P_{\text{uniform}}<0.001$). There was no relationship with the epicardial fiber direction ($R_N=0.53$; $P_{\text{uniform}}=0.20$). In the persAF group, the direction of anisotropy of conduction likelihood showed no relationship with the endocardial bundle direction ($R_N=0.47$; $P_{\text{uniform}}=0.11$) but a good correlation with the epicardial fiber direction ($R_N=0.61$; $P_{\text{uniform}}<0.01$; see Table 2 for mean angle differences).

### Heterogeneity in Anisotropy of Conduction and Bundle Anatomy

In Figure V in the online-only Data Supplement, example maps of anisotropy of CV and anisotropy of conduction likelihood are given (dark to light color indicates increasing anisotropy). The local direction of both types of conduction anisotropy is indicated by arrows (light blue). The corresponding HI and the HI of the endocardial and epicardial bundle patterns are summarized in Table II in the online-only Data Supplement.

No significant differences in HI of anisotropy of CV and in HI of anisotropy of conduction likelihood were found between aAF and persAF. However, the HI of anisotropy of conduction likelihood was significantly lower than the HI of anisotropy of CV for both groups. The HI of the epicardial bundle pattern was higher than the HI of the endocardial bundle pattern for persAF, but not for aAF.

### Discussion

AF persistence goes hand in hand with an increasing complexity in the fibrillatory process. With AF remodeling, the number of fibrillation waves and transmural conduction (epicardial breakthrough) increases, resulting in more complex AF propagation patterns (Figure 3). Anisotropy of conduction is arrhythmogenic, contributing to both initiation and perpetuation of AF. Nevertheless, studies addressing the roles of the complex 3D atrial anatomy in fibrillatory conduction during AF are scarce. Our study demonstrates that during several months of AF in the goat, differences in endo-epicardial bundle direction increase.

In nonremodeled atria, epicardial fibrillation waves propagated fastest along the direction of endocardial bundles, with little influence of epicardial fiber orientation on CV and conduction likelihood of epicardial fibrillation waves. However, in structurally remodeled atria, the conduction of epicardial fibrillation waves was fastest along epicardial fibers. In such atria, endo-epicardial dissociation of electrical activity has occurred, and, therefore, epicardial conduction is determined less by the endocardial bundle network.

### Anisotropy of CV

Low epicardial macroscopic anisotropy of CV during sinus rhythm and pacing has been reported in experimental and clinical studies. Houben et al reported low anisotropy of CV in the human RA during AF (median 1.2). Here, we also report low macroscopic anisotropy of CV during AF. To our surprise, the anisotropy ratio was similar in both remodeled and nonremodeled atria (mean 1.5), indicating that an increase in anisotropy of CV as such is not required to enhance AF stability in remodeled goat atria.

Epicardial anisotropy of CV varies between regions in the atria, partly because of differences in underlying histology. For example, epicardial activation in the LA free wall is relatively uniform with anisotropy ratios of 1.4 to 1.5. Most of the LA endocardium consists of a smooth wall composed of overlapping layers of differently aligned myocardial fibers, with major changes in fiber orientation from the epicardial to
In contrast, epicardial propagation in the posterior LA exhibits marked CV anisotropy, ranging from 1.8 to 3.2. Overall, regional variability in fiber orientation, together with tight electrical coupling between the fibers, leads to a lower degree of anisotropy when conduction is observed on a macroscopic scale.

Interestingly, there is a pronounced discrepancy between these macroscopic findings and reports of high anisotropy at the microscopic level, which ranges from 4.5 to 9.8. Spach et al were the first to demonstrate highly anisotropic propagation in human atrial bundles. Not only the development of microscopic collagenous septa but also the redistribution of gap junctions toward cell ends lead to reduced lateral electrical coupling between myocytes, leading to tortuous conduction pathways in the transverse direction while the longitudinal conduction may be unaffected. Furthermore, fibrosis-induced tissue discontinuities can cause electrical source-to-sink mismatch along these pathways, contributing to rate-dependent local slowing of conduction or conduction block. Finally, AF can be associated with fibroblast proliferation and differentiation into myofibroblasts. Heterocellular electrotonic coupling of myofibroblasts and myocytes has been shown in native atrial tissue and is thought to increase heterogeneity in excitability, refractoriness, and electrical load, potentially inducing heterogeneous slowing of conduction. These mechanisms may also contribute to microscopic zigzag conduction, allowing re-entry to occur in regions as small as 1 to 2 mm, largely facilitating reentrant arrhythmias.

Rearrangement of Bundle Anatomy and Direction of Fastest Conduction

With the transition of aAF to persAF, the orientation differences between epicardial and endocardial bundles in goats become larger. This alteration in atrial bundle architecture might be related to progressive atrial dilatation, occurring during prolonged AF in humans and goats (as much as 12% dilatation during the first 5 days). Chronic atrial dilatation and stretch in fibrillating atria are caused by an elevation in LA pressure and increased wall stress and loss of atrial contractility, resulting in enhanced atrial compliance. As shown in Figure 4C and 4D, atrial dilatation itself may lead to rotation of epicardial fibers with respect to endocardial bundles, leading to a more perpendicular arrangement. This change would be most likely to occur in the thin epicardial layer, rather than in the endocardial bundles that are relatively thick and firmly anchored to the macroanatomy.

Atrial bundle rearrangement has consequences for the observed behavior of fibrillation waves. The alignment of the direction of anisotropy of CV with the endocardial and epicardial bundle directions showed marked differences in aAF and persAF. In nonremodeled atria, the direction of the fastest conduction was mainly dominated by the direction of the endocardial bundles (Figure 5A), in agreement with previous studies demonstrating that lines of block occur parallel to pectinate muscles in sheep and rabbit. This suggests that the overall activation pattern is mainly determined by the endocardial bundle network and that this network is electrically well connected to the epicardial layer.

However, after 7 months of AF, the direction of anisotropy of CV is primarily determined by the epicardial fiber...
orientation (Figure 5D). Earlier studies from our group demonstrated that perpetuation of AF in goat is associated with increased heterogeneity in connexin 40 expression, an increase in intermyocyte distance (endomysial fibrosis) and myocyte hypertrophy. Increased interstitial fibrosis underlies electrical uncoupling of side-to-side connections between neighboring muscle bundles, potentially leading to electrical dissociation within the epicardial layer but more importantly between the epicardial layer and the endocardial bundle network. This is in keeping with a recent report by Verheule et al showing that AF-induced endomysial fibrosis is much more pronounced in the outer millimeter of the atrium than in the deeper layers. In this study, a computer simulation of endo-epicardial dissociation and conduction showed that fibrosis exclusively occurring in the epicardial layer was sufficient to increase electrical dissociation and the complexity of AF.

Putative AF Mechanisms and Atrial Anatomy
Although the role of ectopic activity originating in the pulmonary veins is well established as a mechanism of paroxysmal AF, persistent AF mechanisms are still under debate. Both experimental and human studies suggest rotor and ectopic activity to drive persistent AF. However, detailed high-density direct contact mapping studies in goat and humans identified multiple wavelets, increasing longitudinal dissociation and transmural conduction (epicardial breakthrough) as dominant AF mechanisms. In these studies, stable rotors were rare and not sustained, and breakthroughs were largely because of transmural conduction rather than ectopic activity. The present study was not designed to clarify the mechanism of AF. Rather, our data provide insight into how bundle anatomy affects fibrillatory conduction in the atria, independent of the mechanism driving AF.

To date, studies relating AF propagation to the underlying 3D atrial anatomy are scarce. Dyssynchrony of electrical activation between the epicardial and the endocardial layer was first demonstrated by Schuessler et al. Interestingly, the observed electrical endo-epicardial differences were larger in the thick trabeculated part of the RA. Eckstein et al confirmed these findings and reported that endo-epicardial dissociation increases with AF-induced remodeling. Yamazaki et al performed simultaneous epi/endoepicardial optical mapping and identified atrial scroll waves, transmural rotors that form and meander around regions of sharp transition in myocardial thickness, suggesting that wall thickness variability is an important factor for AF stabilization. Also in humans, fibrillatory conduction is thought to be influenced by the underlying anatomy. Allessie et al reported interwave conduction block to be predominantly oriented parallel to the large pectinate bundles. Narayan et al suggested sustained rotors and repetitive focal beats as drivers of AF; however, the relationship with underlying atrial anatomy has not yet been studied. Our results may help to better understand the relationship between fibrillatory conduction and 3D atrial anatomy. In nonremodeled atria with extensive coupling between all layers, the large endocardial bundles prevail over thin epicardial fibers as pathways for the fastest propagation of fibrillation waves. In remodeled atria, in contrast, loss of endo-epicardial coupling caused by structural remodeling allows epicardial fibrillation waves to primarily propagate along the epicardial muscle bundles. Because during the process of AF the latter become oriented more perpendicularly to the endocardial muscle bundles, epicardial fibrillation waves also preferentially propagate perpendicularly to the endocardial muscle bundles. Because endocardial fibrillation waves conduct along the large muscle bundles, the change in the atrial bundle architecture further enhances endo-epicardial dissociation and thereby the 3D character of the histoanatomic AF substrate.

Relationship Between the 2 Aspects of Conduction Anisotropy
Anisotropy of conduction likelihood provides an additional measure of conduction anisotropy. In our study, the degree of anisotropy of conduction likelihood was strongly reduced in goats with persAF compared with goats with aAF. This observation is in agreement with the increase in complexity of AF propagation patterns over time as reported in this study and others. As more and narrower waves simultaneously meander over the epicardial surface, preferential pathways are less likely to coexist.

Intuitively, it may seem obvious that the direction of the fastest conduction would also be the most frequently encountered conduction direction. However, neither in aAF nor in persAF a strong correlation between the direction of the fastest and the most likely conduction was found. Potentially this is because of the low HI of anisotropy of conduction likelihood. As such, no strong correlation between the direction of anisotropy of conduction likelihood at a certain electrode and the surrounding electrodes exists. In contrast, the HI of anisotropy of CV is 2 to 3x higher than the HI of anisotropy of conduction likelihood and, more importantly, comparable with the HI of epicardial fiber orientation. This supports the hypothesis that conduction likelihood depends more on the macroscopic atrial anatomy, whereas CV is determined by microscopic fiber orientation. In agreement with this, we found the correlation between the direction of fastest conduction with the underlying bundle direction to be larger than the match between the direction of bundles and most likely conduction.

Limitations
Caution is warranted when extrapolating results on the relationship between conduction anisotropy and atrial anatomy from goat to humans. For example, human RA shows a strongly parallel orientation between endocardial bundles, whereas in goat RA endocardial trabeculae follow highly variable directions. However, we think that our general conclusions still hold. These include the observation that endocardial bundle anatomy and epicardial fiber orientation are important determinants of both the velocity and the likelihood of conduction of fibrillation waves and that the extent and the directionality of conduction anisotropy may change with progressive structural remodeling of the atria and electrical dissociation.

Clinical Relevance
Although this study focuses on the relationship between atrial bundle orientation and anisotropy of conduction, the general
conclusions are relevant for clinical practice, in particular the mechanisms contributing to domestication of persAF.

We propose that interindividual differences in AF substrate, as observed in humans, are partly caused by differences in tissue architecture and that in an individual patient, AF characteristics are also determined by the unique underlying atrial anatomy.

It is obvious that the strong influence of the underlying atrial anatomy on fibrillatory conduction demonstrated in this study is relevant for the development of computer models of AF and vice versa (as predictions from structure to function benefit immensely from quantitative modeling). Our data show that not only electrophysiological characteristics but also a detailed anatomy of endocardial and epicardial muscle bundles should be implemented in realistic computer models for AF, in particular if they are to be individualized.

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Disclosures
None.

References

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**CLINICAL PERSPECTIVE**

The architecture of the atrial wall is highly complex and characterized by thick trabeculated endocardial bundles branching out into a thin subepicardial layer. The identified correlation between the atrial bundle anatomy and the atrial fibrillatory conduction pattern has several clinical implications. In particular, our observations help to understand the occurrence of endo-epicardial dissociation of electrical activity and transmural conduction during atrial fibrillation. The study provides evidence that endocardial bundle anatomy and epicardial fiber orientation are important determinants of anisotropic conduction of fibrillation waves. Because in remodeled atria epicardial bundles are oriented perpendicular to the larger endocardial bundles, fibrillation waves follow different paths in the 2 layers. Furthermore, observed interindividual differences in atrial fibrillation substrates in humans can partly be attributed to differences in the individual atrial anatomy, next to differences in electrophysiological properties, clinical risk factors, or remodeling-induced structural alterations such as fibrosis. Finally, our study emphasizes the importance of implementing a detailed anatomy of endocardial and epicardial muscle bundles in computer models for atrial fibrillation, at least if these models are developed for prediction of the efficacy of antiarrhythmic drug therapy or atrial fibrillation ablation.
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SUPPLEMENTAL MATERIAL

I. Supplemental Methods

2.1. Open chest experiments and tissue harvesting

*Animal Model of AF:* An atrial endocardial pacemaker lead (Medtronic Capsurefix®) was implanted in the right atrial appendage in all animals. In the persAF group, the pacemaker lead was connected to an implanted neurostimulator (Medtronic Itrel®) and AF was maintained by repetitive 50Hz burst pacing at 3 times threshold (first 6 weeks 1s on/1s off, remaining weeks 1s/min). Anesthesia was induced with thiopental (10mg/kg, Inresa Arzneimittel GmbH/Germany) and further maintained using Sufentanyl (6µg/kg/h, Hameln/Germany), Midazolam (0.8 mg/kg/h, Actavis/Iceland) and Pancuronium (0.3 mg/kg/h, Organon/The Netherlands). All animal procedures conformed to US National Institutes of Health guidelines and were approved by the local ethical committee of Maastricht University.

*Process of mechanically fixing the mapping array to the underlying atrial tissue.* At the end of recording, the mapping array (supplemental Figure 1A) was mechanically fixed to the underlying left atrial tissue using 13 injection needles. The needles were inserted through holes in a transparent frame surrounding the mapping array. The mapping array was then pulled out of the transparent frame and the whole heart was excised. In supplemental Figure 1B, the excised heart and the frame mechanically fixed to the underlying left atrial tissue is depicted. Hereafter, the free wall bounded by the frame was carefully removed from the rest of the left atrium. The result is depicted in supplemental Figure 1C, which shows the endocardial aspect of the tissue underneath the recording area kept in position by the needles. Then, a counter-frame, with matching dimensions and holes to accept the needles, was placed on the endocardial side by sliding over the needles, thereby aligning counter-frame and
frame. Finally, needles were removed one by one and replaced by sutures. As a result, frame and counter-frame were tied together (see Figure 1A in main manuscript).

2.2. MRI acquisition protocol

*Magnetic Resonance Image Acquisition*. Anatomical magnetic resonance imaging (MRI) of fixed tissue samples, held in the fixating frame and positioned in an MRI tube using low-melting point agar, was performed using a vertical-bore, 11.7T (500Mhz) MR system with a Bruker Avance console (Bruker Medical, Ettingen, Germany) running Paravision 2.1.1, a 40-mm quadrature-driven birdcage coil (Rapid Biomedical, Würzburg, Germany) and a 3D fast gradient echo sequence (TE/TR 1.8/15ms; 15° pulse; field of view 40x40x40 mm, matrix size 512x512x512; voxel size 78 μm x 78 μm x 78 μm; 10 averages; total acquisition time 11h). While MRI resolution (78 μm x 78 μm x 78 μm) is not sufficient to resolve individual myocytes, it is sufficient to identify atrial myobundles.

2.3.1. Analysis of anisotropy of conduction during AF

*Analysis of fibrillation electrograms*. Local activation times were identified by the steepest negative deflection of the electrogram [-dV/dt]_max_. Individual fibrillation waves were delineated by boundaries of conduction block (CV<20cm/s). Two types of waves were identified: ‘peripheral waves’ and ‘epicardial breakthroughs’. For each activation at each electrode, a plane was fitted to activation times at neighboring electrodes belonging to the same wave (maximum square of 5x5 electrodes). The fitted plane indicates local direction of propagation (orientation of the plane) and CV (reciprocal value of the steepness of the plane).

*Threshold of conduction block*

For the identification of the fibrillation waves in the analysis of AF electrograms, the threshold for conduction block was set to CV<20cm/s. To investigate the sensitivity of the
computation of the CV direction to the conduction block threshold, the fibrillation waves and CV were determined at different conduction block thresholds. The difference in conduction velocity direction (in degrees) between directions calculated with the default conduction block threshold of 20 cm/s and lower thresholds of 15 cm/s, 10 cm/s and 5 cm/s was negligible in most cases (see Supplemental Table 3). The median absolute difference (p50) for 15 cm/s, 10 cm/s and 5 cm/s was 0, 0 and 2 degrees respectively. The number of direction vectors calculated to compare with the default conduction block threshold was 79200. These data demonstrate that there is a very small effect of the threshold for block on the vector direction.

Anisotropy of conduction during AF.

Anisotropy values were calculated as follows:

- Anisotropy of conduction velocity. For each electrode, an ellipse was fitted (minimizing the algebraic distance) through all conduction vectors at that electrode during the 4s recording (see supplemental Figure 2A). The degree of anisotropy of conduction velocity was calculated as the ratio of the major axis and the minor axis of the fitted ellipse. The direction of anisotropy of conduction velocity was calculated as the angle between the major axis of the fitted ellipse and the horizontal axis.

- Anisotropy of conduction likelihood. For each electrode, anisotropy of conduction likelihood was quantified based on the circular distribution of conduction vectors. Diametrically opposed conduction vectors are considered equal because they are oriented in the same direction, relatively to myocardial fiber axis. Therefore, conduction vectors were transposed to a range of 0° to 180°. Of these transposed vectors, the circular mean ($\beta_{\text{circ}}$) and variance ($\sigma^2_{\text{circ}}$) were calculated. The degree of anisotropy of conduction likelihood was calculated as $1 - \sigma^2_{\text{circ}}$ [value between 1 (maximal degree of anisotropy of conduction likelihood) and 0 (no degree of anisotropy of conduction likelihood)]. For the direction of
anisotropy of conduction likelihood, the circular mean was used (see supplemental Figure 2B).

For the total degree of anisotropy of conduction velocity and anisotropy of conduction likelihood per goat, all the degrees of anisotropy of conduction velocity and anisotropy of conduction likelihood determined at each electrode were averaged per goat. For the purpose of standardization and to exclude directions at electrodes with low and thus uncertain degree of anisotropic conduction, only directions of electrodes with a degree of anisotropy of conduction velocity \( \geq 1.5 \) and a degree of anisotropy of conduction likelihood \( \geq 0.20 \) were included in the analysis. These cut-off values have been chosen so that on average \( \sim 51\% \) (min. 22\%, max. 87\%) of all vectors were included in the analysis. The technique used is capable of detecting differences between the direction of fastest conduction (anisotropy of conduction velocity) and the direction of the most likely conduction (anisotropy of conduction likelihood, see supplemental Figure 3 for a schematic example).

II. Supplemental Results.

3.3. Direction of endocardial bundles versus epicardial fiber orientation.

In addition to differences between the direction of endocardial bundles and the direction of epicardial fibers in aAF and persAF, we further analyzed directional differences between epicardial fibers within the epicardial layer for aAF and persAF and differences between the endocardial bundles within the endocardial layer (Supplemental Figure 6). To illustrate the differences, results should be compared to figure 4 in the main manuscript. As mentioned in the main manuscript, epicardial fibers are oriented more perpendicularly to endocardial bundles in persAF (\( \mathbf{R}_N=0.19, \ P_{\text{uniform}}<0.001, P<0.05 \) vs. aAF, Figure 6B) than in aAF (\( \mathbf{R}_N=0.44, \ P_{\text{uniform}}<0.001, \) Figure 6A). Likewise, when comparing epicardial fibers within the epicardial layer, no differences were present between aAF (\( \mathbf{R}_N=0.96, \ P_{\text{uniform}}<0.001, \) Figure 6C) and persAF (\( \mathbf{R}_N=0.96, \ P_{\text{uniform}}<0.001, \) Figure 6D). When comparing endocardial bundles
within the endocardial bundle network, no differences were present between aAF ($R_N=0.95$, $P_{\text{uniform}}<0.001$, Figure 6E) and persAF ($R_N=0.99$, $P_{\text{uniform}}<0.001$, Figure 6E). Also, within the endocardial layer the differences in bundle direction were smaller than between the epicardial and the endocardial bundles. These results clearly illustrate that during seven months of AF in the goat, endo-epicardial differences in direction of the bundles increase but that this is not the case within the epicardial and endocardial layers.
III. Supplemental Tables

Supplemental Table 1.

Mean degree of anisotropy of conduction during pacing and AF. *P<0.001 aAF versus persAF; †P<0.01 pacing versus AF; NA=not applicable

<table>
<thead>
<tr>
<th></th>
<th>Degree of anisotropy of conduction velocity</th>
<th>Degree of anisotropy of conduction likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>aAF - Pacing (n=6)</td>
<td>1.68±0.19</td>
<td>NA</td>
</tr>
<tr>
<td>persAF - Pacing (n=4)</td>
<td>1.85±0.23</td>
<td>NA</td>
</tr>
<tr>
<td>aAF - AF (n=6)</td>
<td>1.55±0.07</td>
<td>0.39 ± 0.05</td>
</tr>
<tr>
<td>persAF - AF (n=5)</td>
<td>1.51±0.07†</td>
<td>0.20 ± 0.02*</td>
</tr>
</tbody>
</table>
Supplemental Table 2.

Heterogeneity index of direction of anisotropy of conduction and heterogeneity index of direction of atrial bundles for the aAF group and the persAF group. *P<0.05 between direction of anisotropy of conduction velocity and anisotropy of conduction likelihood. †P<0.05 between direction of epicardial and endocardial bundles.

<table>
<thead>
<tr>
<th>Heterogeneity Index</th>
<th>aAF (n=6)</th>
<th>persAF (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direction of anisotropy of conduction velocity</td>
<td>18.8±9.2</td>
<td>28.2±16.6</td>
</tr>
<tr>
<td>Direction of anisotropy of conduction likelihood</td>
<td>9.3±1.7*</td>
<td>8.4±2.4*</td>
</tr>
<tr>
<td>Direction of epicardial bundles</td>
<td>17.7±4.2</td>
<td>15.7±2.3</td>
</tr>
<tr>
<td>Direction of endocardial bundles</td>
<td>18.2±3.8</td>
<td>13.0±1.8†</td>
</tr>
</tbody>
</table>
Supplemental Table 3.

Percentiles of the angle differences (in degrees) of the vectors calculated with the default conduction block threshold of 20 cm/s compared to lower thresholds of 15 cm/s, 10 cm/s and 5 cm/s.

<table>
<thead>
<tr>
<th>Threshold</th>
<th>5%</th>
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<th>15%</th>
<th>20%</th>
<th>25%</th>
<th>30%</th>
<th>35%</th>
<th>40%</th>
<th>45%</th>
<th>50%</th>
<th>55%</th>
<th>60%</th>
<th>65%</th>
<th>70%</th>
<th>75%</th>
<th>80%</th>
<th>85%</th>
<th>90%</th>
<th>95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 cm/s</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.32</td>
<td>7.13</td>
<td>13.20</td>
<td>20.81</td>
<td>30.54</td>
<td>43.21</td>
<td>58.62</td>
<td>79.38</td>
<td>105.12</td>
<td>138.18</td>
<td></td>
</tr>
<tr>
<td>10 cm/s</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.10</td>
<td>5.62</td>
<td>11.73</td>
<td>20.31</td>
<td>33.31</td>
<td>52.75</td>
<td>89.38</td>
<td></td>
</tr>
<tr>
<td>15 cm/s</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0.07</td>
<td>5.31</td>
<td>13.86</td>
<td>28.34</td>
<td>58.62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
IV. Supplemental Figures and Figure Legends

Supplemental Figure 1.

A 256-channel square mapping array used to record epicardial potentials.

A. Contact-side of the mapping array (256 channels, interelectrode distance 1.5mm, array dimensions 22.5 x 22.5 mm). The array is shown inserted into a transparent frame. Green arrows point at holes used subsequently for attachment of the frame to the tissue.

B. Epicardial aspect of an excised heart, showing the transparent frame attached to the atrial tissue by 13 injection needles (e.g. green arrows, corresponding to locations in panel A).

C. Endocardial aspect of the excised tissue with points of the injection needles clearly visible.
Supplemental Figure 2.

A. Diagram of the quantification of anisotropy of conduction velocity for an individual electrode (red dot). An ellipse is fitted through 7 conduction vectors ($R_1=$major axis, $R_2=$minor axis). The ratio $R_1/R_2$ is the degree of anisotropy of conduction velocity and $\alpha$ the direction of anisotropy of conduction velocity.

B. Quantification of anisotropy of conduction likelihood using the same 7 hypothetical conduction vectors. All conduction vectors are projected onto a circular scale from $0^\circ$ to $180^\circ$ ($\beta_{\text{circ}}=$circular mean, $\sigma^2_{\text{circ}}=$circular variance). The degree of anisotropy of conduction likelihood was calculated as $1$-circular variance ($\sigma^2_{\text{circ}}$). The direction of anisotropy of conduction likelihood is calculated as the mean of angles (circular mean=$\beta_{\text{circ}}$).
Supplemental Figure 3. Schematic examples of direction of anisotropy of conduction velocity and anisotropy of conduction likelihood. Hypothetical conduction vectors (black arrows) during 4s of AF at a single electrode (red point) are depicted.

A. Calculation of the direction of anisotropy of conduction velocity and anisotropy of conduction likelihood will result approximately in the same direction (purple).

B. Most small conduction vectors are orientated along the same axis, but a few large vectors are orientated along an axis perpendicular to the first axis. This will result in a direction of anisotropy of conduction velocity (blue) that is different from the direction of anisotropy of conduction likelihood (green).
Supplemental Figure 4. Spread of absolute angle differences (range 0° to 90°) between direction of anisotropy of conduction velocity and anisotropy of conduction likelihood during acute AF (A) and persistent AF (B). Ratio=ratio small angle differences/(small + large angle differences); mean= mean angle difference; $P_{uniform}$=test for uniform distribution, N=number of observations. See text for further explanation.
Supplemental Figure 5. Example maps of anisotropy of conduction. One map consists of 16x16 squares corresponding to electrode positions on the mapping array. Dark to light color indicates increasing anisotropy (see reference bar). Direction of anisotropy of conduction velocity and anisotropy of conduction likelihood is indicated (blue arrows).
Supplemental Figure 6. Absolute angle differences (range 0º to 90º) between direction of epicardial and endocardial bundles during aAF (A) and persAF (B), within the epicardial layer during aAF (C) and persAF (D) and within the endocardial layer during aAF (E) and persAF (D). Differences in bundle direction are larger between the epicardial layer and the
The differences in bundle direction between the epicardial layer and the endocardial layer increase with the persistence of AF. No differences between aAF and persAF are observed within the epicardial or the endocardial layer. Ratio = ratio small angle differences/(small + large angle differences); mean = mean angle difference; $P_{\text{uniform}}$ = test for uniform distribution, N = number of observations.

V. Supplemental References


VI. Supplemental movie

The movie shows an example of the original high-resolution 3D magnetic resonance imaging (MRI) data-sets used for determining the direction of endocardial bundles and epicardial fibers. The upper part of the tissue is the epicardial layer, the lower part is the endocardial bundle network. During the movie, the image sequence starts at the left atrial appendage and progresses over the left atrial free wall towards the inter-atrial septum. In the first part of the movie, an epicardial layer is visible at both the upper and the lower edge of the tissue, representing the left atrial appendage.