Intramyocardial Adiposity After Myocardial Infarction: New Implications of a Substrate for Ventricular Tachycardia

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Background—Collagen has been attributed as the principal structural substrate of ventricular tachycardia (VT) after myocardial infarction (MI), even though adiposity of myocardium after MI is well recognized histologically. We investigated the effects of intramyocardial adiposity compared with collagen on electrophysiological properties, connexin43 expression, and VT induction after MI.

Methods and Results—Simultaneous left ventricular plunge-needle, noncontact mapping was performed in sheep without MI (MI−; n=5), with MI and inducible VT (MI+VT+; n=7), and with MI and no inducible VT (MI+VT−; n=8). Histological intramyocardial quantity of adipose and collagen and degree of discontinuity were coregistered with electrophysiological parameters (MI+; 290 specimens). Additional assessment of connexin43 expression was performed. Left ventricular scar contained a body mass–independent abundance of adipocytes (adipose:collagen=0.8). Increased adipose density and discontinuity contributed to a greater inverse correlation (r) with conduction velocity (r for adipose=0.39, r for discontinuity=0.45, r for collagen=0.26) and electrogram amplitude (r for adipose=0.73, r for contiguity=0.77, r for collagen=0.68) compared with collagen. Collagen density was similar between the MI+ groups (P>0.29). However, the MI+VT+ group demonstrated a significant (all P≤0.01) increase in adipose (8%) and discontinuity (qualitative) and decrease in conduction velocity (13%) and electrogram amplitude (21%) at MI borders compared with the MI+VT− group. In scar, myocytes adjacent to fibrofatty interfaces demonstrated increased connexin43 lateralization. A gradient increase in adipose was observed at sites that supported preferential presystolic VT activation and exhibited attenuation of excitation wavelength (P<0.001).

Conclusions—Intramyocardial adiposity, in association with myocardial discontinuity within left ventricular scar borders, is a significant factor associated with altered electrophysiological properties, aberrant connexin43 expression, and increased propensity for VT after MI. (Circulation. 2013;128:2296-2308.)

Key Words: adipose tissue ◼ cicatrix ◼ connexin 43 ◼ electrophysiology ◼ myocardial infarction ◼ scar ◼ tachycardia, ventricular

Histologic evidence of intramyocardial adipose has been observed in relation to left ventricular (LV) myocardial scars in explanted hearts in 68% of patients with ischemic heart disease1 and in 84% of patients with a history of myocardial infarction (MI).2 The deposition of fat within the myocardium after MI has been postulated to be involved in the healing cascade after MI. This remodeling process, called lipomatous metaplasia, continues for several months to years after MI and is structurally characterized by the replacement of collagen by interstitial adipocytes.1

With the recent use of imaging techniques such as magnetic resonance imaging and computed tomography, findings similar to those initially described by Baroldi et al1 and Su et al2 have been observed noninvasively in isolated case reports3–10 and larger subgroups of MI survivors.11–14

Earlier studies examining the nature of arrhythmogenesis indicate that fibrosis has been the primary structural observation associated with ventricular tachycardia (VT).15 The principal mechanism was slowing of conduction as a result of the presence of collagen fibers acting as a barrier against electrophysiological propagation. Paradoxically, not all patients develop VT after MI despite the presence of intramyocardial...
collagen. In other conditions associated with the development of VT such as arrhythmogenic dysplasia, the widespread infiltration of myocardium by adipose is well documented.

To date, the effects of interstitial adipose on electrophysiological properties and the propensity for VT after MI have not been reported. In this study, we determined whether the distribution and quantity of interstitial adipose present in the LV after MI were associated with electrophysiological changes and affected the propensity for VT induction.

**Methods**

The procedures followed were in accordance with the National Health and Medical Research Council of Australia and were approved by the Animal Ethics Committee of Westmead Hospital.

**Preparation of Animals Used for the Study**

Experiments were performed on 22 castrated male sheep weighing 45±8 kg. All animals were placed on a diet of lucerne hay (standardized by mean flock weight) as the main source of nutrients that was administered at regular feeding times during the day. The sheep were allowed to supplement feed ad libitum on a toxic plant–free pasture and were given freedom to roam on an equal area of land.

During the experiments, the sheep were sedated with intramuscular xylazine (0.5 mg/kg), and anesthesia was induced with an intravenous bolus of propofol (4 mg/kg) before intubation. General anesthesia was maintained with 1% to 4% isoflurane in 100% oxygen, and intravenous NaCl (100 mL/h) was maintained throughout the procedure. A total of 5 sheep were used as electrophysiological mapping controls (MI−). Anterior MIs were created in the remaining 17 sheep. General anesthesia was induced and maintained with as the main source of nutrients that was administered at regular feeding times during the day. The sheep were allowed to supplement feed ad libitum on a toxic plant–free pasture and were given freedom to roam on an equal area of land.

After a 30-minute settling period, endocardial pacing at a cycle length of 400 milliseconds was performed from at least 7 different locations.

**Data Collection**

After a 30-minute settling period, endocardial pacing at a cycle length of 400 milliseconds was performed from at least 7 different locations.
spanning the entire LV in addition to the right ventricular apex. The electrophysiological study was then repeated.

During each study, unipolar electrograms from each of the plunge-needle electrodes were recorded on the Prucka Cardiolab system (GE Healthcare) using a filter band pass of 0.05 to 500 Hz (1-kHz sampling). Noncontact reconstructed electrograms from 2048 endocardial sites were simultaneously recorded with the Ensite system using a filter band pass of 0.1 to 300 Hz (1.2-kHz sampling). A synthesized timing signal (in-house) was simultaneously recorded on both systems for offline temporal alignment of contact and noncontact electrograms.

At the completion of the study, the sheep were euthanized, and numbered markers were sutured at each needle location. The entire heart was excised and fixed in 10% formalin. In a subset of animals, transmural blocks (≈1×1 cm; n=90) were excised from random needle sites and bisected transmurally. One-half of the specimen was frozen in liquid nitrogen and stored at −70°C, and the other half was stored in 10% formalin at room temperature.

**Histology**

After formalin fixation (2 weeks), a total of 290 transmural blocks of myocardium (≈1×1 cm) surrounding each needle were excised, dehydrated with 100% ethanol, and embedded in paraffin wax. A 5- to 8-μm-thick section was cut from each paraffin block and stained with Gomori trichrome (Figure 1A). For colocalization analysis, frozen tissue blocks were consecutively sectioned at a thickness of 8 to 10 μm, stained with Gomori trichrome and Oil Red O, and labeled with anti-connexin43 (Cx43; Figure 2). Each tissue section was then digitally scanned (Scope CS, Aperio Technologies; or Nanozoomer, Hamamatsu, Japan) at ≥20 objective magnification and imported into in-house customized analysis software.

As previously described, the software was able to differentiate between myocardium and collagen in scar on the basis of a color threshold algorithm for red and blue pixels, respectively (Figure 1). The software was also able to identify the tissue boundary and to calculate its area.

Adipocytes were observed as areas characteristically exhibiting fibrous interlobular septa with Gomori trichrome staining. These structures were confirmed from Oil Red O–stained specimens as capable of compartmentalizing neutral lipids (Figure 2A2 and 2A3). Quantification of adipose within Gomori trichrome specimens involved a multistep algorithm described as follows: (1) identification of tissue space by subtraction of blue and red pixels, 2-dimensional gaussian low-pass filtering of tissue space and conversion to binary color, and identification of adipocyte compartments by subtraction of objects with >2800 pixels and having an interconnectivity neighborhood of <8 pixels (Figure 1). In addition, the area of epicardial adipose was easily identified and excluded from measurement by tagging of this area with an operator-dependent polygon measurement tool. The areas of viable myocardium, collagen matrix, and intra-myocardial adipose tissue were calculated as a percentage of the total intramyocardial tissue area.

A qualitative approach was used to assess myocardial contiguity. For each specimen, the myocardial structure and state of viable contiguity were graded on a scale of 1 to 5 by 3 independent histologists. Agreement between observers was first assessed on a random test data set of 30 histological specimens before all specimens were scored. The scoring criteria were as follows: 1=remodeling is observed (presence of collagen/adipose) and viable myocardium is not contiguous or does not contain viable myocardium; 2=remodeling is observed and viable myocardium is partially contiguous; 3=remodeling is observed, the viable myocardium is contiguous, and additional isolated bundles of viable myocardium are present; 4=remodeling is observed and the injured tissue is confined to a small area of the specimen; and 5=no remodeling is observed.

Immunofluorescence was performed on a subset of frozen specimens (n=60) to highlight the distribution of nuclei, mitochondria, and total Cx43 expression associated with fibrofatty infiltration of myocardium (Figures 2 and 3). Sections cut to 5-μm thickness were fixed with 4% (wt/vol) paraformaldehyde and permeabilized with 0.1% (vol/vol) Triton-X 100, and nonspecific sites were blocked with 2% (vol/vol) goat serum. Diluted mouse anti-Cx43 monoclonal primary antibody (1:250, Chemicon) was applied overnight at 4°C. Diluted Alexa 488–conjugated F(ab’)2 fragment of goat anti-mouse secondary antibody (1:1000; Invitrogen) was applied for 2 hours. Nucleic acids were counterstained, and slides were mounted on glass coverslips with Prolong Gold antifade reagent with DAPI (Life Technologies). Sections were imaged with Nanozoomer or confocal fluorescent microscopy (Olympus). For each specimen, a 200-μm grid was overlaid on the digital scan, and at each grid space, the interface between myocytes and collagen, adipose, or mixed collagen and adipose was labeled. The distance from each interface site (n=141) to the nearest myocyte cluster expressing Cx43 at the intercalated disks was measured (Figure 3D–3G).

**Data Processing**

The electrograms were exported for offline analysis on customized software developed with Matlab (version 7.11, Mathworks). Electrogram amplitude was defined as the QRS peak-peak deflection, and dV/dtmin was defined as the steepest descending slope of the unipolar electrogram. For VT activation mapping, the fiducial point was defined as the earliest dV/dtmin that occurred within the presystolic
part of the VT cycle. Activation time was defined as the interval from the fiducial point to the local dV/dtmin.

Various models of conduction velocity (CV) estimation, each specific to the modality of measurement, spatial density, and regularity of the measurement field, have been described previously.19,20 We adopted a hybrid approach as follows: Activation time measurements from plunge-needle electrodes were spatially interpolated by distance-weighted moving averaging onto a 2048-point cloud of the LV, which was acquired with the Ensite mapping system. Points outside the needle grid were not interpolated. For each point of the interpolated activation map, the steepest gradient (vector) of conduction within a 10-mm radius was determined by calculating the site of earliest and latest activation at 2-mm intervals from the central point (Figure 4). The CV was then calculated using least-squares regression of that vector. Vectors with values of |r|<0.95 were excluded.

Activation recovery interval calculation, which is an estimation of the functional refractory period, was based on the method described by Yue et al.21 Wavelength of excitation (λ), calculated as CV times activation recovery interval, was reported as uncorrected (λ and λcorrected) and corrected (λcomp) values based on endocardial LV surface area.

All local electrophysiological criteria recorded during multisite pacing were averaged before statistical analysis.

**Figure 4.** Method used to calculate conduction velocity using multielectrode plunge needles. **Left,** Preferential pathway of conduction recorded with multielectrode plunge needles. ← indicates steepest gradient of conduction; ●, interpolated grid; solid lines, isochrones; and dashed circles, distance from the central point at 2-mm intervals. **Right,** Calculation of local conduction velocity at the central data point as a function of the slope of the linear regression line. d indicates distance from central data point; and |Δt|, absolute difference in activation time along the preferential path of conduction from the central data point.

**Statistical Analysis**

An independent-samples t test was conducted for pair-wise comparisons of growth characteristics between the MI+VT− and MI+VT+ groups (Table 1).

The relationships between substrate characteristics (viability, contiguity, collagen density, and intramyocardial adipose density) and electrophysiological criteria (amplitude, dV/dtmin, CV, and λ) were investigated with the Spearman rank correlation coefficient (Table 2). Correlations were calculated individually within each sheep and summarized as the mean correlation coefficient. One sample t tests were used to test for significant departure from zero for within-sheep rank correlations. Standardized mean difference effect size was calculated with the Hedges g statistic based on differences in the within-group correlations and pooled-group correlations.22

Histological samples were categorized as low, below-average, above-average, and high viability based on cutoff values of 31% (−1 SD), 57% (mean), and 83% (1 SD). Electrogram parameters (amplitude, dV/dtmin, CV, and λ) and myocardial structure (contiguity index, collagen percent, intramyocardial adipose percent) were then aggregated into mean values stratified by viability category for each sheep. Electrophysiological parameters were log transformed to stabilize the variance before analysis. The statistical software package S-Plus version 6.1 (Insightful Corp, Seattle WA) was used to fit linear mixed effects models to these data. The linear mixed effects models were used to assess the within-subject association of the effects of viability and disease status (MI+VT− and MI+VT+) on electrogram parameters (Table 3). Additional analysis with linear mixed effects (within-subject) was used to compare the means of each of the electrogram criteria across the MI−, MI+VT−, and MI+VT+ groups for the high-viability category only and to assess the myocardial structural characteristics of the presystolic to late-systolic VT activation pathways (from contact multielectrode plunge-needle data). In the linear mixed effects models, when appropriate, individual sheep were random effects, animal groups were fixed effects, viability categories were both fixed and random effects, and the interaction term was viability category times animal group.

The effect of scar interface type and the nearest distance to the zone of normal Cx43 expression at the intercalated disks of myocytes was observed relative to interface type. Color bar index associated with D through F. #Adipocytes.

**Results**

**Electrophysiology of Lipomatous Metaplasia**

Sustained monomorphic VT was inducible in 7 of 15 animals after MI. The ability to induce or not induce monomorphic sustained VT lasting >10 seconds during the electrophysiological...
study was reproducible in all cases at the time of the mapping studies. The time span between electrophysiology studies did not differ significantly between groups (Table 1). A total of 12 VT morphologies (1.7 morphologies per animal) with similar cycle lengths (electrophysiology study, 207±35 milliseconds; mapping, 253±47 milliseconds; \( P = 0.860 \)) requiring the same number of extrastimuli (electrophysiology study, 3.4±0.5; mapping, 3.4±0.6; \( P = 0.584 \)) were induced between follow-ups.

### Post–MI Remodeling Results in Fibrofatty Infiltration of the Myocardium

Histological assessment using 2 differential staining techniques identified the presence of adipose tissue and collagen at all intramural layers of the myocardium (Figure 2). Both collagen matrix and adipose were colocalized within infarct territory exhibiting low to below-average density of viable tissue. At these sites, adipose was highly abundant, with an intramyocardial adipose:collagen ratio of \( \approx 0.8 \) at the time of

### Table 1. Between-Group Comparison of Animal Growth Characteristics and Time Interval Between Studies

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Group</th>
<th>MI+VT−</th>
<th>MI+VT+</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time, wk</td>
<td>Procedure MI induction to EPS</td>
<td>7.2 (4 to 55)</td>
<td>7.8 (5 to 53)</td>
<td>0.974</td>
</tr>
<tr>
<td></td>
<td>EPS to mapping study</td>
<td>68 (2 to 124)</td>
<td>75 (4 to 160)</td>
<td>0.189</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>MI induction</td>
<td>49 (45 to 55)</td>
<td>47 (37 to 55)</td>
<td>0.288</td>
</tr>
<tr>
<td></td>
<td>EPS study</td>
<td>49 (46 to 55)</td>
<td>49 (37 to 56)</td>
<td>0.437</td>
</tr>
<tr>
<td></td>
<td>Mapping study</td>
<td>57 (38 to 94)</td>
<td>58 (45 to 84)</td>
<td>0.761</td>
</tr>
<tr>
<td>Growth rate, g/wk</td>
<td>MI induction to mapping study</td>
<td>200 (−83 to 538)</td>
<td>200 (72 to 293)</td>
<td>0.844</td>
</tr>
</tbody>
</table>

Data are expressed as median (range). \( P \) value is based on comparison between the MI+VT− and MI+VT+ groups. EPS indicates electrophysiological study; MI, myocardial infarction; and VT, ventricular tachycardia.

### Table 2. Correlations of Histological and Electrophysiological Criteria

<table>
<thead>
<tr>
<th>Electrophysiological Criteria</th>
<th>Histological Criteria</th>
<th>( r )</th>
<th>( P )</th>
<th>( g )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude</td>
<td>Viability</td>
<td>0.74±0.12</td>
<td>&lt;0.01</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Contiguity</td>
<td>0.77±0.11</td>
<td>&lt;0.01</td>
<td>2.16</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>−0.68±0.20</td>
<td>&lt;0.01</td>
<td>−2.12</td>
</tr>
<tr>
<td></td>
<td>Interstitial Adipose</td>
<td>−0.73±0.12</td>
<td>&lt;0.01</td>
<td>−0.06</td>
</tr>
<tr>
<td>( \Delta t_{\text{exit}} )</td>
<td>Viability</td>
<td>−0.69±0.10</td>
<td>&lt;0.01</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>Contiguity</td>
<td>−0.70±0.12</td>
<td>&lt;0.01</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>0.65±0.16</td>
<td>&lt;0.01</td>
<td>−2.18</td>
</tr>
<tr>
<td></td>
<td>Interstitial Adipose</td>
<td>0.64±0.09</td>
<td>&lt;0.01</td>
<td>−1.30</td>
</tr>
<tr>
<td>CV</td>
<td>Viability</td>
<td>0.42±0.19</td>
<td>&lt;0.01</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Contiguity</td>
<td>0.45±0.21</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>−0.26±0.41</td>
<td>0.53</td>
<td>−0.25</td>
</tr>
<tr>
<td></td>
<td>Interstitial Adipose</td>
<td>−0.39±0.19</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>Viability</td>
<td>0.44±0.24</td>
<td>&lt;0.01</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Contiguity</td>
<td>0.47±0.24</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>−0.43±0.25</td>
<td>0.08</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Interstitial Adipose</td>
<td>−0.39±0.28</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>( \lambda ) (MI+VT−)</td>
<td>Viability</td>
<td>0.44±0.25</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Contiguity</td>
<td>0.47±0.28</td>
<td>0.09</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>−0.48±0.24</td>
<td>0.08</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Interstitial Adipose</td>
<td>−0.32±0.35</td>
<td>0.21</td>
<td>−0.44</td>
</tr>
<tr>
<td>( \lambda ) (MI+VT+)</td>
<td>Viability</td>
<td>0.44±0.25</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Contiguity</td>
<td>0.47±0.28</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Collagen</td>
<td>−0.37±0.26</td>
<td>0.08</td>
<td>−0.28</td>
</tr>
<tr>
<td></td>
<td>Interstitial Adipose</td>
<td>−0.46±0.18</td>
<td>0.15</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Values are mean±SD. CV indicates conduction velocity; \( g \), measure of effect magnitude; \( \lambda \), wavelength of excitation; MI, myocardial infarction; \( r \), mean within-sheep rank correlation; and VT, ventricular tachycardia.
mapping (Figure 5). At sites remote from the infarct border, intramyocardial adipose was less abundant and confined to vascular tissue.

The morphological appearance and diameter (≈50 μm) of cardiac-derived adipocytes were similar in normal hearts and within areas of scar, except adipose derived from scar was surrounded by collagen matrix (Figure 3A and 3B). The phenotype of adipocytes within scar was confirmed to be white adipose as a result of the unilocular and monovacuolar appearance of the cell and confinement of nucleic acids (nuclei, mitochondria) to the cytoplasmic cell membrane (Figure 3C). Intracellular accumulation of neutral lipid as present in adipocytes was not significantly observed within myocytes with the current methods (Figure 2).

The relationships between the presence of collagen, adipose, viable myocardium, and contiguity are illustrated in Figure 5. There was a moderate correlation between the presence of collagen and adipose (r=0.574, P<0.01). The presence of collagen (r=−0.913, P<0.01) and adipose (r=−0.800, P<0.01) was inversely correlated with myocardial viability. A similar inverse relationship was observed with myocardial contiguity (collagen, r=−0.729; adipose, r=−0.827; all P<0.01).

Table 3. Comparison of Electrophysiological Criteria Between Groups With Respect to Viability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Myocardial Viability</th>
<th>MI−</th>
<th>MI+VT−</th>
<th>MI+VT+</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude, mV</td>
<td>High</td>
<td>21.5±14.6</td>
<td>13.1±2.5*</td>
<td>13.1±3.3*</td>
<td>0.987</td>
</tr>
<tr>
<td></td>
<td>Above average</td>
<td>14.2±6.2</td>
<td>9.2±4.4</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Below average</td>
<td>9.0±6.5</td>
<td>5.1±4.5</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>4.4±3.0</td>
<td>3.9±2.7</td>
<td></td>
<td>0.549</td>
</tr>
<tr>
<td>dV/dt&lt;sub&gt;min&lt;/sub&gt;</td>
<td>High</td>
<td>−0.21±0.15</td>
<td>−0.16±0.08</td>
<td>−0.16±0.05</td>
<td>0.952</td>
</tr>
<tr>
<td></td>
<td>Above average</td>
<td>−0.23±0.18</td>
<td>−0.13±0.10</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Below average</td>
<td>−0.12±0.08</td>
<td>−0.06±0.04</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>−0.07±0.04</td>
<td>−0.05±0.02</td>
<td></td>
<td>0.038</td>
</tr>
<tr>
<td>Local CV, m/s</td>
<td>High</td>
<td>0.91±0.42</td>
<td>0.74±0.22</td>
<td>0.68±0.27*</td>
<td>0.589</td>
</tr>
<tr>
<td></td>
<td>Above average</td>
<td>0.83±0.28</td>
<td>0.64±0.35</td>
<td></td>
<td>0.084</td>
</tr>
<tr>
<td></td>
<td>Below average</td>
<td>0.66±0.28</td>
<td>0.56±0.16</td>
<td></td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.54±0.19</td>
<td>0.51±0.17</td>
<td></td>
<td>0.613</td>
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<tr>
<td>λ</td>
<td>High</td>
<td>242±76</td>
<td>233±77</td>
<td>211±69*</td>
<td>0.247</td>
</tr>
<tr>
<td></td>
<td>Above average</td>
<td>267±85</td>
<td>199±100</td>
<td></td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>Below average</td>
<td>199±87</td>
<td>174±48</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>169±54</td>
<td>169±47</td>
<td></td>
<td>0.161</td>
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<tr>
<td>λ Corrected</td>
<td>High</td>
<td>265±73</td>
<td>197±54*</td>
<td>189±78*</td>
<td>0.258</td>
</tr>
<tr>
<td></td>
<td>Above average</td>
<td>226±73</td>
<td>181±110</td>
<td></td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Below average</td>
<td>175±83</td>
<td>146±43</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>150±50</td>
<td>132±50</td>
<td></td>
<td>0.407</td>
</tr>
</tbody>
</table>

The P value is based on a comparison of the MI+VT− and MI+VT+ groups. Values are mean±SD. CV indicates conduction velocity; λ, wavelength of excitation; MI, myocardial infarction; and VT, ventricular tachycardia.

*P<0.05 based on comparisons with the MI− group.

Figure 5. Comparison of structural properties of myocardium between the myocardial infarction (MI) groups with noninducible ventricular tachycardia (MI+VT−) and with inducible monomorphic VT lasting >10 seconds (MI+VT+) relative to myocardial viability. Collagen content did not differ between these groups, whereas significant impairment of myocardial contiguity and increased intramyocardial adipose were observed within the infarct border zone (areas exhibiting below- or above-average viability) of VT+ animals.
Collagen Plays a Subordinate Role in Altering Electrophysiological Properties of Myocardium

When we compared the correlations for each of the histological and electrophysiological criteria measured, we determined that increased collagen content did not contribute to reductions in electromgram amplitude, dV/dt\text{min}, and CV to the extent that decreased contiguity, decreased contiguity, and increased adipose did (Table 2). This is further reinforced by the small effect size reported for collagen compared with each of the other histological criteria. In fact, the correlation between collagen density and CV was not significant, whereas significance was achieved with the other histological criteria.

In contrast, viability, collagen, and adipose quantity were not significantly associated with $\lambda$ when the groups were combined. However, separate group analysis indicated that adipose was the only significant determinant of $\lambda$ in the MI+VT+ group, whereas significance was not achieved in this respect in the MI+VT− group for any of the histological criteria measured.

Overall, the combined effect magnitude (summation of effect sizes) of each histological criteria, in order of greatest to lowest electrophysiological influence, was interstitial, contiguity ($g=4.13$), viability ($g=2.26$), adipose ($g=-1.52$), and collagen ($g=-4.55$).

Furthermore, we can confirm that very low interobserver variability was present between pathologists in the assessment of myocardial contiguity ($r=0.935$, $P<0.001$).

The Area of Abnormal Gap Junctional Remodeling Between Myocytes Increases in the Presence of Intramyocardial Adipose

We did not observe evidence of Cx43-dependent coupling between adipocytes (Figure 3C). Sites remote from the infarct site exhibited normal expression of Cx43 at the intercalated disks of myocytes (Figure 2C and 2C2). Within scar, however, the Cx43 expression pattern varied, depending on myocyte scar interface structure. Lateralized Cx43 expression in myocytes was observed at 80.5%, 81.1%, and 93.6% of sites where the myocyte interface contained collagen, adipose, and mixed collagen-adipose, respectively (Figure 2 and 2B2). Similarly, the mean distance between the interface and area of normal Cx43 expression at the intercalated disks was greater for sites that contained adipose compared with collagen (Figure 3D–3G). Interface sites that contained both adipose and collagen exhibited significantly greater impact in increasing the zone of Cx43 lateralization in scar than interface sites that contained collagen alone.

Arrhythmogenic Propensity Is Associated With Altered Structural and Electrophysiological Remodeling

Healed MI resulted in global electrophysiological remodeling, whereas structural remodeling was confined predominantly to the LV apex, anterior apical wall, apical-mid interventricular septum, and right ventricular apex. In comparisons of electrophysiological properties within areas of high myocardial viability between the MI− and MI+ groups, significant reductions in amplitude and CV were observed in the VT-inducible group (Table 3).

Comparisons between the MI+VT− and MI+VT+ groups revealed significant structural and electrophysiological differences within the scar border spanning the above-average to below-average viability zones (Table 3 and Figure 5). Altered remodeling in the MI+VT+ group involved reduced electromgram amplitude, reduced electromgram $dV/dt_{\text{min}}$, slower CV, and shorter $\lambda$ (corrected and uncorrected; Table 3). These electrophysiological changes were associated with decreased myocardial contiguity and increased intramyocardial adipose content (Figure 5). Interestingly, there was no difference in LV collagen density between the MI+VT− and MI+VT+ groups with respect to viability (Figure 5). This finding was consistent with a similar endocardial low-amplitude area observed between groups, which was defined with arbitrary cutoffs of 30% (MI+VT−, 4.1±5.3 cm$^2$; MI+VT+, 5.7±6.8 cm$^2$; $P=0.274$) and 50% (MI+VT−, 12.2±4.9 cm$^2$; MI+VT+, 15.0±8.9 cm$^2$; $P=0.200$) of the global maximum peak-peak amplitude respectively.

Furthermore, no significant difference in epicardial adipose was observed between the MI+VT− and MI+VT+ groups (range, 0.3% to 0.6%; $P>0.05$).

Remodeling Continues Over Time Independently of Body Weight

During the course of the experiments, there were no significant differences in body weight or growth rates between the MI+VT− and MI+VT+ groups (Table 1). However, chronic-phase remodeling across all MI+ groups continued over study periods of up to 25 months (Figure 6). This was characterized as a linear reduction of intramyocardial collagen and a linear increase in adipose content over time, which were confined only to areas with low to below-average viability. Body weight did not correlate with these changes.

Furthermore, myocardial contiguity was also uninfluenced by body weight. However, high temporal variation within scar borders was the contributing factor to the low correlations observed in relation to infarct maturity.

Structural Characteristics of the Arrhythmogenic Circuits

The VT activation sequence was elucidated in 6 of 7 animals (11 morphologies). The remaining animal had an unmappable VT despite having a relatively slow cycle length of 268 milliseconds.

To examine how structural changes after MI are distributed within reentrant circuits, we compared the structural characteristics of myocardium at various time points spanning presystolic (≤5% of the VT cycle) to late-systolic (>20% of the VT cycle) activation sites during VT propagation (Figure 7A–7D).

In 6 animals, collagen density was homogenously distributed within these circuits (Figure 7E). At the earliest detectable sites of systolic activation during VT, intramyocardial adipose density was significantly greatest, whereas myocardial contiguity was significantly reduced (Figure 7F and 7G). Specifically, a linear inverse relationship between intramyocardial adipose content and VT activation time was observed, whereas a remarkable difference in myocardial contiguity was evident between sites activating within ≤5% of the VT cycle.
and late-systolic activating sites (Figure 7F and 7G). Overall, myocardium within early sites of VT activation was composed of approximately equal quantities of intramyocardial adipose and collagen and bordered by a thin rim of conducting myocardium that was at least partially contiguous. The preferential path of propagation remote from presystolic sites involved activation of myocardium that is more contiguous and is less infiltrated by intramyocardial adipose. Noncontiguous myocardium was not observed to be located within the reentrant circuits.

Discussion

From this study, we confirm that lipomatous metaplasia, as described initially by Baroldi et al1 and later in other studies,2,11–13,23 of patients with healed MI is recapitulated in the ovine model. In light of the study aims, novel findings from this study are 4-fold. First, increased local intramyocardial adiposity and increased myocardial discontinuity are associated with significantly altered local electrophysiological properties and slowing of conduction. In contrast, increased density of the local collagen matrix imparts a secondary and less significant influence in this respect. Second, induction of VT is associated with altered and progressive structural remodeling, culminating in increased adiposity of the infarct border zones. Third, intramyocardial adiposity is significantly and inversely associated with excitation wavelength in animals with an increased propensity for VT. Fourth, sites that are likely to be critical in the stability and formation of reentrant circuits are traversed by a thin band of conducting tissue and consist of significantly increased intramyocardial adipose compared with less critical sites within the reentrant circuits.

Our study had several strengths. First, electrophysiological measurements and quantification of adipose and collagen were automated to eliminate observer bias. To complement this, we adopted a conventional validated method for qualitative assessment of tissue structure to estimate myocardial contiguity. We did not attempt to automate quantification of this tissue property because of the mathematical complexity in assessing structural heterogeneity when the scale relationship of the macroscopic and microscopic structure of each specimen was variable. Second, the presence of intramyocardial adipose was confirmed independently with 2 differential histological staining methods used to identify the location of lipid molecules and lipid compartments. Third, coregistration of histological and electrophysiological data was performed with intramural plunge-needle mapping, which provided a

Figure 6. Effect of infarct maturity and body weight on collagen content, intramyocardial adipose content, and index of myocardial contiguity. In areas of low to below-average viability, increased infarct maturity correlated directly with increased adipose content and inversely with collagen content. This association was not related to animal weight. Furthermore, contiguity index was not influenced by infarct maturity or body weight. MI indicates myocardial infarction.
stable electrode-myocardial interface. Assessment of VT inducibility was confirmed on 2 separate occasions during the study. Moreover, activation mapping of the VT reentrant circuits with plunge-needle electrodes was complemented by global, high-density, noncontact activation mapping.

The pattern of interstitial fibrosis and preserved tissue anisotropy at the scar periphery is present in both human and animal infarcts. Histological analysis of human myocardial specimens obtained during subendocardial resection for the surgical cure of VT has revealed that the arrhythmogenic anatomic substrate includes viable myocytes dissociated by collagen, producing loss of intracellular connections and the development of thin, discontinuously connected myocardial fibres. This classic model has long been established as the cause of conduction slowing with the assumption that conduction traverses in a zigzag pattern through small myocardial channels within scar. Our data support the principal involvement of conducting channels in reentrant circuits on the premise that the early sites of reentrant activation situated within the substrate borders were at least partially contiguous. However, in divergence from the classic model, we demonstrated that slowing of conduction, which is a prerequisite for reentry, is not exclusively altered by collagen.

On the basis of multiple recent clinical reports and case studies, the presence of intramyocardial adipose in patients with previous MI may be underestimated. According to those investigations, lipomatous metaplasia occurs in ≈15% to 89% of patients with MI, depending on the disease progression and modality of assessment used. On the basis of those studies, histological assessment has greater sensitivity than magnetic resonance imaging– and computed tomography–based assessment of myocardial adipose. Lipomatous metaplasia is more prevalent in patients with left coronary artery infarcts involving the anterior, septal, and lateral walls of the LV apex. Furthermore, intramyocardial adipose is found in greater density within the infarct territory and increases with time from the index of ischemic insult. In this ovine model, coronary artery occlusion was performed

Figure 7. Spatiotemporal relationship of ventricular tachycardia (VT) reentrant circuits to pathological features of the left ventricle (LV) after myocardial infarction (MI). A, Example of global LV presystolic to late systolic propagation during VT in 1 animal with noncontact mapping. Three-dimensional geometry orientation: projection, anterior; apex, inferior; and septum, left. Corresponding 2-dimensional projections of inferior half of the LV chamber are shown with superimposed isochrones. B through D, Spatial distribution of collagen, intramyocardial adipose, and index of myocardial contiguity in the same animal based on histology. Gray areas represent sites where needle registration and histology could not be performed because of limited access from the epicardium. E through G, Summary of collagen quantity, intramyocardial adipose quantity, and index of myocardial contiguity in all animals (MI with inducible monomorphic VT lasting >10 seconds) relative to VT activation time measured with intramural plunge-needle electrode contact mapping. ★ Earliest activation site.
with the intent to create consistent infarcts that parallel the predominant infarct territory of patients having the greatest risk of developing lipomatous metaplasia. This consistency resulted in similarly sized infarcts between animals as interpreted from voltage mapping and visualization of the electroanatomic maps.

Investigations from a large, multicenter trial (Multicenter Automatic Defibrillator Implantation Trial II [MADIT II]) revealed that in patients with ischemic LV dysfunction, obesity was associated with an increased risk of ventricular arrhythmias resulting in appropriate device therapy independently of diabetes mellitus.\textsuperscript{24} In contrast, we found that animal growth rate was not a significant factor in the modulation of the VT substrate properties. All animals were fed the same diet, were of the same sex, had ample access to pasture for exercise, and were exposed to identical environmental conditions. In agreement with our results, multiple independent studies with smaller patient cohorts than the MADIT II trial have been unable to demonstrate a relationship between body mass index and the prevalence of lipomatous metaplasia.\textsuperscript{11–14}

Speculation has arisen from clinical observations as to whether adipose alters electrophysiological properties of the myocardium and whether adipose contributes to reentrant tachycardias.\textsuperscript{25} In support of this, a large scar volume is not critical for the development of life-threatening arrhythmias in arrhythmogenic right ventricular dysplasia patients with ventricular adiposity.\textsuperscript{26} Indeed, our own observations suggest that intramyocardial adipose significantly impedes myocardial conduction and attenuates both electrogram amplitude and slope to a greater degree than the presence of collagen. There are additional properties of the substrate, beyond the scope of this study, that may contribute to this phenomenon. Electric resistance is much greater for adipose than myocardium, whereas the opposite is true for myocardium with high collagen density.\textsuperscript{27,28} In light of this, we can confirm that adipocytes from within scar do not couple via Cx43 proteins. However, observations from other studies have demonstrated that human preadipocytes are able to express other ion channels ($I_{\text{KCa}}$, $I_{\text{CCa}}$) to participate in cell proliferation.\textsuperscript{29} Therefore, adipose tissue, because of its high resistance and ionic membrane properties, may act as a current sink, dampening the electrotonic interactions that occur between sparse neighboring myocytes at infarct borders. From our data, we believe this current sink effect may extend locally beyond adipose-rich scar borders by a distance factor of 45% to 85% above that observed for collagen-rich borders on the basis of increased Cx43 lateralization in adipose-bordering myocytes.

Although these data do not confirm lipotoxicity as a mechanism, such source-sink effects may contribute to attenuation of electrogram amplitude, suppression of electrogram slope, and slowing of conduction. Electrotonic coupling across collagen fibers between neighboring myocytes has been observed at the microscopic scale.\textsuperscript{30,31} This mechanism of passive conduction may not necessarily reduce CEs, electrogram amplitude, or slope but would contribute to electrogram fractionation. In our study, this is further compounded by relatively normal gap junctional coupling observed between myocytes that are present within scar borders but are not in proximity to adipose.

Adipose tissue possesses both endocrine and paracrine effects and is a source of inflammatory mediators.\textsuperscript{32,33} At present, there is a paucity of information on the direct influence of adipose tissue on myocardium. One investigation demonstrated that adipokines, in particular fatty acid-binding protein 4 secreted by mature human adipocytes, were able to suppress contractility of rat cardiomyocytes.\textsuperscript{34} Although no studies have examined the direct electrophysiological consequence of the effect of adipose-derived factors on myocardium, it is plausible from other studies that suppression of myocyte contractility, in addition to reduced myocardial structural support resulting from infiltration by adipose, can lead to altered mechano-electric feedback in the tissue.\textsuperscript{35} Such altered mechano-electric feedback is likely to manifest as ventricular ectopic beats early in the evolution of VT circuits, and their persistence and frequency are likely to be involved in the ventricular remodeling process.\textsuperscript{35,36}

Induction of VT was highly reproducible in this study spanning the continual remodeling process, indicating that the arrhythmogenic conditions were present within the early healed phase of MI. Consistent with other studies, VT systolic activation was earliest at the substrate border zones in all animals with inducible VT.\textsuperscript{37} At these locations, the arrhythmogenic group exhibited significantly decreased myocardial contiguity and increased adipose content, both of which were strongly associated with local slowing of conduction and attenuation of excitation wavelength. Furthermore, the combined presence of adipose and fibrotic tissue is the cause of reduced myocardial contiguity observed in this model. However, the degree of myocardial contiguity did not change temporally as a result of the relative relationship between adipose proliferation and collagen catabolism. This process has been shown to be an integral mechanism of adipocyte differentiation and involves the expression of matrix metalloproteinase factors by the surrounding medium, which in turn degrade collagen.\textsuperscript{38,39}

It is well known that tissue structure, organization, and function contribute to the dynamics of myocardial propagation. For reentry to occur, 2 conditions must be satisfied: local conduction block and availability of sufficient time for the depolarizing wave front to travel around an area of block so that excitability of the tissue within proximity to the line of block can be restored. The wavelength of excitation fundamentally describes the distance an activation wave front has traveled during its refractory period.\textsuperscript{40,41} When the wavelength of excitation is long, a large area of block is required to sustain reentry. Conversely, for shorter wavelengths, resulting from either slowing of conduction or short repolarization time, smaller areas of block are sufficient to support reentry.

Our data show that wavelength shortening was functionally dependent on CV, which was attributed to increased structural disarray. Our data strongly support this mechanism of reentry because excitation wavelength was significantly attenuated within substrate borders of animals with inducible VT, even after correction for LV surface area. These VT anchor sites were in concordance with areas demonstrating
earliest presystolic activity during VT and exhibited a high degree of adiposity.

Further research is warranted to determine whether intramyocardial adiposity is a byproduct of preestablished reentrant circuits or whether there is sufficient adipogenic early remodeling within the arrhythmogenic substrate to consolidate early formed reentry circuits in the chronic stage of disease. One study of experimental MI demonstrated that lipids released as non–membrane-bound droplets (fatty acid) accumulate in highest intracellular concentrations within the peripheral ischemic border zone. This accumulation occurs as early as 6 hours after coronary artery occlusion and increases progressively to 24 to 48 hours after occlusion. Although this may contribute to long-term remodeling, in our study, we were unable to demonstrate evidence of significant intracellular lipid accumulation in healed MI using the current histological techniques.

Conclusions
The structural remodeling that occurs within the postinfarct border zone differs between animals relative to the propensity of VT. In this study, collagen was not a significant factor in determining the propensity to VT induction. However, an increased intramyocardial adipose density and the presence of narrow myocardial conducting channels were structural proponents that were significantly associated with critical reentrant circuit isthmuses. This combination of structural properties present within the infarct border zone was a significant factor involved in slowing conduction, necessary for the development of reentrant circuits. Therefore, the formation and stability of these circuits were supported functionally by attenuation of the excitation wavelength. Furthermore, the progressive remodeling in this ovine model with lipomatous metaplasia is consistent with reported clinical findings and was not influenced by dietary intake, body weight, or infarct size.

Further research is required into how reentrant circuits become established within the early healing phase in light of characterizing the phenotype, source, and paracrine effects of white adipose on myocyte function and determining whether abrogation of the intramyocardial adipogenic pathway may be of therapeutic benefit in this disease process.

Clinical Relevance
If these findings are confirmed, the assessment of the presence of intramyocardial adipose within the infarct border zone with noninvasive techniques may be applied to predict the propensity for post-MI VT and mortality. Moreover, these findings can be used to develop novel therapeutic agents against cardiac adipogenesis.

Substrate mapping with unipolar peak-peak amplitude may have potential benefit in guiding radiofrequency ablation of lipomatous scar borders with currently available mapping systems.

The presence of adipose may be important in pharmacological agent selection. For instance, amiodarone is a lipophilic compound, suggesting that high concentrations of the drug may accumulate within peri-infarct areas to promote proarrhythmia. Detailed clinical studies on the effect of antidiabetic agents, which are capable of modulating adipogenesis, by selective stimulation of the peroxisome proliferator-activated receptor-γ transcriptional pathway may be warranted in patients after MI.

Furthermore, the presence of adipose may have implications in the efficacy of internal cardioverter defibrillation in which the reentrant circuits involved in the maintenance of ventricular arrhythmias may be electrically insulated from external electrical stimulation or the presence of adipose tissue may lead to the formation of secondary sources during defibrillation.

Although collagen deposition is critical for increasing the structural support of the myocardium, the presence of adipose is likely to reduce structural support. Hence, care must be taken during mapping studies to reduce the risk of catheter-related perforation of the myocardium at sites that are likely to be targeted for ablation because of the increased adipose content.

Limitations
This study was performed in sheep hearts. Despite indications of an accelerated form of lipomatous metaplasia in this study compared with clinical manifestations of the disease, this postinfarct ovine model has been well validated and is representative of postinfarct VT substrate in humans.

Serial assessment of myocardium was not performed in this study because of the limited robustness of available imaging modalities to identify intramyocardial adipose. However, the histological techniques used in this study were able to quantify subtle differences in adipose volume with high-resolution imaging and automated analysis used to eliminate operator error. Although the histological data were derived from 2-dimensional sections, the data represent a 3-dimensional model of myocardium because the orientation of tissue excision and orientation before sectioning were both randomized.

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Disclosures
None.

References
Electrophysiology of Lipomatous Metaplasia


The prevalence of intramyocardial adipose tissue (IMAT) in patients after myocardial infarction is ≈15% to 89%, depending on disease progression and assessment modality. To date, the electrophysiological influence of IMAT in this setting has been undefined. In this large-animal study, we have provided histological and electrophysiological evidence that IMAT is associated with ventricular tachycardia in the chronic phase of myocardial infarction. If these findings are confirmed clinically, noninvasive identification of IMAT within infarct borders may be applied to predict the propensity for ventricular tachycardia and mortality. The “gray zone” on magnetic resonance imaging in the chronic phase of myocardial infarction has been correlated with inducible and spontaneous ventricular tachycardia. It would be of interest to determine whether the gray zone on magnetic resonance imaging correlates with IMAT. Early intervention requiring the development of pharmacological or gene therapies to inhibit adipogenesis after myocardial infarction may be of potential therapeutic benefit to patients. Substrate-guided radiofrequency ablations rely on identification of low-voltage areas to identify scar. Similar principles would be valid for “adipose mapping” of critical scar borders involved in reentry. Myocardial structural support resulting from IMAT may be compromised; hence, care must be taken to reduce the risk of catheter-related perforation. The presence of IMAT may be important in pharmacological agent selection. For instance, lipophilic agents such as amiodarone may accumulate within peri-infarct areas to promote antiarrhythmic or proarrhythmic effects. Detailed clinical studies on the effect of antidiabetic agents, which are capable of modulating adipogenesis, by selective stimulation of the peroxisome proliferator-activated receptor-γ transcriptional pathway may be warranted in post–myocardial infarction patients.
Intramyocardial Adiposity After Myocardial Infarction: New Implications of a Substrate for Ventricular Tachycardia

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