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

Running title: Poulipooulos et al.; Electrophysiology of lipomatous metaplasia

Jim Pouliopoulos, BSc.MSc(Med).PhD1,2; William W.B. Chik, MB.BS.FRACP1,2; Ajita Kanthan, MB.BS, FRACP2; Gopal Sivagangabalan B.Med.Sc, MB.BS, PhD, FRACP1,2; Michael A. Barry, BSc1; Peter N.A. Fahmy, MBChB, FRACP1,2,3; Christine Midekin, MD1; Juntang Lu, BVSc1; Eddy Kizana MB.BS(Hons), PhD, FRACP1,2, 3; Stuart P. Thomas, MB.BS, PhD1,2; Aravinda Thiagalingam, MBBS, PhD, FRACP1,2; Pramesh Kovoor, MB.BS, PhD, FRACP1,2

1Dept of Cardiology, Westmead Hospital, Sydney, Australia; 2The University of Sydney, Sydney, Australia; 3Westmead Millennium Institute, Westmead, Australia

Address for Correspondence:
Jim Pouliopoulos, BSc, MSc (Med), PhD
Department of Cardiology
Westmead Hospital
PO Box 533
Wentworthville, NSW 2145, Australia.
Tel: +61-2-9845-6027
Fax: +61-2-9845-8323
E-mail: jim.pouliopoulos@sydney.edu.au, jim.pouliopoulos@gmail.com

Abstract

Background—Collagen has been attributed as the principle structural substrate of ventricular tachycardia (VT) post-myocardial infarction (MI) even though adiposity of myocardium post-MI is well recognised histologically. We investigated the association of intramyocardial adiposity in comparison to collagen on electrophysiological (EP) properties, connexin-43 (Cx43) expression, and VT induction post-MI.

Methods and Results—Simultaneous, left ventricular (LV) plunge-needle, and noncontact mapping was performed in sheep (a) without MI (MI-, n=5), (b) with MI and inducible VT (MI+VT+, n=7) and (c) with MI and no inducible VT (MI+VT-, n=8). Histological intramyocardial quantity of adipose, collagen, and degree of discontinuity was co-registered with EP parameters (MI+, 290 specimens). Additional assessment of Cx43 expression was performed. LV 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 (CV, r: adipose=0.39, discontinuity=0.45, collagen=0.26), and electrogram amplitude (Vpp, r: adipose=0.73, contiguity=0.77, collagen=0.68), compared to collagen. Collagen density was similar between MI+ groups (p>0.29). However the MI+VT+ group demonstrated a significant (all p<=0.01) increase of adipose (8%) and discontinuity (qualitative); and decrease of CV (13%), and Vpp (21%) at MI borders compared to the MI+VT- group. In scar, myocytes adjacent to fibro-fatty interfaces demonstrated increased Cx43 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 LV scar borders is a significant factor associated with altered EP properties, aberrant Cx43 expression, and increased propensity for VT post-MI.

Key words: adipose tissue, collagen, myocardial infarction, ventricular tachycardia, conduction velocity, connexin43 expression, cardiac gap junction connexins
Introduction

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

With the recent use of imaging techniques such as magnetic resonance imaging (MRI) and computed tomography (CT), similar findings initially described by Baroldi and Su have been observed non-invasively in isolated case reports \(^3\)-\(^10\) and larger sub-groups of MI survivors \(^11\)-\(^14\).

Earlier studies examining the nature of arrhythmogenesis, indicate fibrosis has been the primary structural observation associated with ventricular tachycardia \(^15\). The principal mechanism was attributed to slowing of conduction due to the presence of collagen fibres acting as a barrier against electrophysiological propagation. Paradoxically, not all patients develop ventricular tachyardia (VT) post 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 propensity for VT post-myocardial infarction have not been reported. In this study, we determined whether the distribution and quantity of interstitial adipose present in the left ventricle post myocardial infarction was 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 ± 8kg. All animals were placed on a diet of lucerne hay (standardised by mean flock weight), as the main source of nutrients, which was administered at regular feeding times of the day. The sheep were allowed to supplement feed *ad libitum* on 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 (0.5mg/kg) and anaesthesia was induced with an intravenous bolus of propofol (4mg/kg) prior to intubation. General anaesthesia (GA) was maintained with 1-4% isoflurane in 100% oxygen and intravenous NaCl (100mL/hr) was maintained throughout the procedure. A total of 5 sheep were used as electrophysiological mapping controls (MI-). Anterior myocardial infarctions (MI) were created in the remaining 17 sheep by inflating a 3.0mm angioplasty balloon in the middle of the ovine left anterior descending artery equivalent for 3 hours as described by Reek et al., (1999)¹⁶. A total of 15 animals survived this procedure to undergo electrophysiological studies. Sotalol was administered at an oral dose of 40mg twice daily following MI until the mapping procedure to reduce the risk of fatal arrhythmias. Sotalol was discontinued for over 5 days (mean of 11 half lives) prior to electrophysiological and mapping studies.

An electrophysiological study (EPS) using standardised protocols was performed at >3 weeks post-MI. General anaesthesia was induced and maintained as described for the MI
induction procedure. Induction of VT was attempted using programmed ventricular stimulation from the right ventricular apex with a drive train of 8 paced beats at a cycle length of 400ms and up to a maximum of 4 extrastimuli at twice the diastolic threshold. Each extrastimulus was decremented at 10ms interval until refactoriness. If VT was not inducible after 2 attempts from the right ventricular (RV) apex, the same induction protocol was repeated from the RV outflow tract.

Sheep with non-inducible VT were categorised as controls (MI+VT-), whilst sheep with inducible monomorphic VT lasting longer than 10 seconds were allocated to the VT group (MI+VT+).

Mapping Study

An electrophysiological mapping study was performed at a random time interval, between 3 and 172 weeks post-MI using combined noncontact and contact mapping employing plunge needle electrodes. For control animals, studies were conducted after a 2-week settling period to allow for acclimatisation of the animal to the local paddock and handling regime.

Our group previously described the use of noncontact mapping using the Ensite system in an ovine model with needle electrogram validation. A similar methodology was used for this procedure as follows. A left thoracotomy was performed through the 4th intercostal space. The heart was exposed, and 20 multielectrode (area of each electrode = 3.7mm², interelectrode distances = 1.5mm) plunge needles were inserted via the epicardium in (i) scarred myocardium, (ii) peripheral scar, and (iii) in normal myocardium. Each needle was positioned ~1cm apart from it’s neighbouring needle, spanning the apex, anterior septum, postero-lateral wall and base of the LV. Scar tissue location during needle insertion was identified by visual inspection and palpation. The needle length and corresponding electrode number varied from 2-4 electrodes.
depending on the estimated thickness of myocardium at the site of deployment. The needle electrodes were configured for unipolar recording with the rib retractors connected as the indifferent electrode.

A quadripolar electrophysiology catheter (St Jude Medical, USA) was percutaneously positioned at the right ventricular apex for pacing. The Ensite (V6.1, Endocardial Solutions, USA) multi-electrode array and quadripolar mapping catheter (Navistar, Biosense Webster, USA) were introduced into the left ventricular apex via the femoral artery using a retrograde aortic approach.

The 5.6 kHz Enguide locator signal on the Ensite system was used to collect an endocardial geometry of the left ventricle (LV) during roving of the mapping catheter. Annotation of 3D needle electrode positions were incorporated on the geometry by passing the Enguide locator signal independently through each of the needle electrodes. To eliminate the effect of motion-related artefact during localization of needles, annotation of needle positions on the LV geometry was performed during gating to ventricular systolic activation.

**Data Collection**

After a 30 minute settling period, endocardial pacing at a cycle length of 400ms was performed from at least 7 different locations spanning the entire left ventricle, in addition to the right ventricular apex. An EPS 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, USA) using a filter bandpass of 0.05–500Hz (1kHz sampling). Noncontact reconstructed electrograms from 2048 endocardial sites were simultaneously recorded using the Ensite system using a filter bandpass of 0.1–300Hz (1.2kHz sampling). A synthesised 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 (~1x1 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 stored in 10% formalin at room temperature respectively.

**Histology**

After formalin fixation (2 weeks), a total of 290 transmural blocks of myocardium (~1 cm x 1 cm) surrounding each needle were excised and dehydrated with 100% ethanol, and embedded in paraffin wax. A 5-8 μm-thick section was cut from each paraffin block and stained with Gomori-trichrome ([figure 1A](#)). For co-localization analysis, frozen tissue blocks were consecutively sectioned at a thickness of 8-10 μm, and stained using Gomori-trichrome, Oil-Red-O (ORO), and labelled using anti-Cx43 ([figure 2](#)). Each tissue section was then digitally scanned (scope CS, Aperio Technologies, USA; or Nanozoomer, Hamamatsu, Japan) at 20x objective magnification and imported into in-house customised analysis software.

As previously described, the software was able to differentiate between myocardium and collagen in scar, based on a colour threshold algorithm for red and blue pixels respectively ([figure 1](#)). The software was also able to identify the tissue boundary and calculate its area.

Adipocytes were observed as areas characteristically exhibiting fibrous interlobular septa using gomori trichrome staining. These structures were confirmed from ORO stained specimens as capable of compartmentalising neutral lipids ([figure 2, A2-A3](#)). Quantification of adipose within gomori trichrome specimens involved a multi-step algorithm described as follows: 1) identification of tissue space by subtraction of blue and red pixels; 2) two dimensional Guassian
low-pass filtering of tissue space and conversion to binary color; 3) identification of adipocyte compartments by subtraction of objects with >2800 pixels and having an interconnectivity neighbourhood of <8 pixels (*figure 1*). In addition, the area of epicardial adipose was easily identified and excluded from measurement by tagging of this area using an operator dependent polygon measurement tool. The areas of viable myocardium, collagen matrix, and intramyocardial adipose tissue were calculated as a percentage of the total intramyocardial tissue area.

A qualitative approach was employed for assessment of myocardial contiguity. For each specimen, the myocardial structure and state of viable contiguity was graded on a scale of 1-5 by three independent histologists. Agreement between observers was first assessed on a random test dataset of 30 histological specimens prior to scoring of all specimens. The scoring criteria were as follows: 1 = Remodelling observed (presence of collagen/adipose) and viable myocardium is not contiguous or does not contain viable myocardium; 2 = Remodelling observed, and viable myocardium is partially contiguous; 3 = Remodelling observed, the viable myocardium is contiguous, and additional isolated bundles of viable myocardium are present; 4 = Remodelling observed, and the injured tissue is confined to a small area of the specimen; 5 – No remodelling observed.

Immunofluorescence was performed on a subset of frozen specimens (n=60) to highlight distribution of nuclei, mitochondria, and total connexin-43 (Cx43) expression associated with fibro-fatty infiltration of myocardium (*figure 2 and 3*). Sections cut to 5μm thickness were fixed with 4% (wt/vol) paraformaldehyde, permeabilized with 0.1% (v/v) Triton-X 100, and non-specific sites blocked with 2% (v/v) goat serum. Diluted mouse anti-Cx43 monoclonal primary antibody (1:250, Chemicon) was applied overnight at 4°C. Diluted Alexa488-conjugated F(ab’)_2 fragment of goat anti-mouse secondary antibody (1:1000, Invitrogen) was applied for 2 hours.
Nucleic acids were counter-stained and slides were glass cover-slip mounted using Prolong Gold anti-fade reagent with DAPI (Life Technologies). Sections were imaged using Nanozoomer, or confocal fluorescent microscopy (Olympus). For each specimen, a 200μm grid was overlayed over the digital scan, and at each grid space, the interface between myocytes and 1. collagen, 2. adipose, or 3. mixed collagen and adipose was labelled. The distance from each interface site (n=141) to the nearest myocyte cluster expressing Cx43 at the intercalated disks was measured (figure 3D-G).

Data Processing

The electrograms were exported for offline analysis on customised software developed with the Matlab (V7.11, Mathworks, USA). Electrogram amplitude was defined as the QRS peak-peak deflection and \( \frac{dV}{dt_{\text{min}}} \) was defined as the steepest descending slope of the unipolar electrogram. For VT activation mapping, the fiducial point was defined as the earliest \( \frac{dV}{dt_{\text{min}}} \) that occurred within the pre-systolic part of the VT cycle. Activation time was defined as the interval from the fiducial point to the local \( \frac{dV}{dt_{\text{min}}} \).

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.\textsuperscript{19, 20} We adopted a hybrid approach as follows: Activation time measurements from plunge needle electrodes were spatially interpolated by distance-weighted moving averaging on to a 2048 point-cloud of the left ventricle, which was acquired using the Ensite mapping system. Points outside of the needle grid were not interpolated. For each point of the interpolated activation map, the steepest gradient (vector) of conduction within a 10mm radius was determined by calculating the site of earliest and latest activation at 2mm intervals from the central point (figure 4). The CV was then calculated using least squares regression of that
vector. Vectors with $r<0.95$ were excluded.

Activation recovery interval (ARI) calculation which is an estimation of the functional refractory period was based on the method described by Yue et al, (2004). Wavelength of excitation ($\lambda$) was calculated as $CV \times ARI$, and was reported as uncorrected ($\lambda$) and corrected ($\lambda_{corrected}$) values based on endocardial LV surface area.

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

**Statistical analysis**

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

The relationship between substrate characteristics (viability, contiguity, collagen density, and intramyocardial adipose density) and electrophysiological criteria (amplitude, $dV/dt_{min}$, $CV$, and $\lambda$) was investigated using Spearman’s rank correlation coefficient (table 2). Correlations were calculated individually within each sheep and summarised as the mean correlation coefficient. One sample T-tests were used to test for significant departure from zero within-sheep rank correlations. Standardised mean difference effect size was calculated using Hedges' $g$ statistic ($g$) based on differences in the within-group correlations and pooled-group correlations.

Histological samples were categorised as low, below average, above average, and high viability based on cut-off values of 31% (-1SD), 57% (mean), and 83% (+1SD). Electrogram parameters (amplitude, $dV/dt_{min}$, $CV$, and $\lambda$) and myocardial structure (contiguity index, collagen%, intramyocardial adipose%) were then aggregated into mean values stratified by viability category for each sheep. Electrophysiological parameters were log transformed to
stabilise the variance prior to analysis. The statistical software package S-Plus Version 6.1 (Insightful, Corporation, Seattle USA) was used to fit linear mixed effects (LME) models to these data. The LME 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 using LME (within-subject) was used to compare 1) the means of each of the electrogram criteria across MI-, MI+VT- and MI+VT+ groups for the high viability category only, and 2) to assesss the myocardial structural characteristics of the pre-systolic to late-systolic VT activation pathways (from contact multielectrode plunge needle data). In the LME models, where appropriate, individual sheep were random effects, animal groups were fixed effects, viability category were both fixed and random effects, and the interaction term was viability category × animal group.

The effect of scar interface type and nearest distance to the zone of normal intercalated disk Cx43 expression was assessed using ANOVA (figure 3).

**Results**

**Electrophysiology study**

Sustained monomorphic ventricular tachycardia was inducible in 7/15 animals post-MI. The ability to induce or not induce monomorphic sustained VT lasting >10 seconds during the EPS 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/animal) with similar cycle lengths (electrophysiology study 207±35ms; mapping 253±47ms, *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-myocardial infarct remodelling results in fibro-fatty infiltration of the myocardium

Histological assessment using two 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 co-localised 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 ~0.8 at the time of 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 (~50μm) of cardiac derived adipocytes were similar in normal hearts, and within areas of scar, with the exception that adipose derived from scar was surrounded by collagen matrix (figure 3A,B). The phenotype of adipocytes within scar was confirmed to be white adipose due to 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 using the current methods (figure 2).

The relationship between the presence of collagen, adipose, viable myocardium and contiguity is 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 $p<0.01$).

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 electrogram amplitude, dV/dt_{min}, and CV, to the extent that decreased contiguity, decreased viability, and increased adipose did (table 2). This is further reinforced by the small effect size reported for collagen compared to 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 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 regard, 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-lowest electrophysiological influence, were 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 inter-observer variability was present between pathologists in the assessment of myocardial contiguity (\( r=0.935, p<0.001 \)).

The area of abnormal gap junctional remodelling between myocytes increases in the presence of intramyocardial adipose

We did not observe evidence of Cx-43 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, C2). 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 B2). 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 to collagen (figure 3D-G). 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 remodelling.**

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

Comparisons between MI+VT- and MI+VT+ groups, revealed significant structural and electrophysiological differences within the scar border spanning the above average to below average viability zone (table 3, and figure 5). Altered remodelling in the MI+VT+ group involved reduced electrogram amplitude, reduced electrogram dV/dtmin, slower CV, and shorter λ (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 left ventricular collagen density between MI+VT- and MI+VT+ groups with respect to viability (figure 5). This finding was consistent with similar endocardial low amplitude area observed between groups, which were defined using arbitrary cut-offs of 30% (MI+VT- 4.1±5.3cm²; MI+VT+ 5.7±6.8cm², p=0.274) and 50% (MI+VT- 12.2±4.9; MI+VT+ 15.0±8.9cm², p =0.200) of the global maximum peak-peak amplitude.
respectively.

Furthermore, no significant difference in epicardial adipose was observed between MI+VT- and MI+VT+ groups (range = 0.3 to 0.6%, \( p \) >0.05).

**Remodelling continues over time, independent of body weight.**

During the course of experiments, there were no significant differences in body weight or growth rates between MI+VT- and MI+VT+ groups (*table 1*). However, chronic-phase remodelling across all MI+ groups continued over study periods of up to 25 months (*figure 6*). This was characterised as a linear reduction of intramyocardial collagen and 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 6/7 animals (11 morphologies). The remaining animal, had an un-mappable VT despite having a relatively slow cycle length of 268ms.

In order to examine how structural changes post-MI are distributed within reentrant circuits, we compared the structural characteristics of myocardium at various time points spanning pre-systolic (\(<= 5\% \) of VT cycle) to late-systolic (\(>20\% \) of VT cycle) activation sites during VT propagation (*figure 7A-D*).

In 6 animals, collagen density was homogenously distributed within these circuits (Figure 7E). At the earliest detectable sites of systolic activation during VT, we identified, intramyocardial adipose density was significantly greatest, whereas myocardial contiguity was
significantly reduced at the earliest detectable sites of systolic activation during VT (Figure 7F-G). 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 \( \leq 5\% \) of the VT cycle and late systolic activating sites (Figure 7F-G). Overall, myocardium within early sites of VT activation were composed of approximately equal quantities of intramyocardial adipose and collagen, bordered by a thin rim of conducting myocardium that was at least partially contiguous. The preferential path of propagation remote from pre-systolic sites involved activation of myocardium that is more contiguous, and is less infiltrated by intramyocardial adipose. Non-contiguous myocardium was not observed to be located within the reentrant circuits.

### Discussion

From this study, we confirm that “lipomatous metaplasia”, as initially described by Baroldi et al., and in other studies, of patients with healed myocardial infarction, is recapitulated in the ovine model.\(^1,2,11-13,23\) In light of the aims, novel findings from this study are fourfold. 1) Increased local intramyocardial adiposity and increased myocardial discontinuity is 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. 2) Induction of ventricular tachycardia is associated with altered and progressive structural remodelling culminating in increased adiposity of the infarct border zones. 3) Intramyocardial adiposity, is significantly and inversely associated with excitation wavelength in animals with an increased propensity for ventricular tachycardia. 4) Sites which 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 to less critical sites within the reentrant circuits.

Our study had several strengths. Primarily, electrophysiological measurements and quantitation 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 quantitation of this tissue property, due to mathematical complexity in assessing structural heterogeneity when the scale-relationship of the macroscopic and microscopic structure of each specimen was variable. Secondly, the presence of intramyocardial adipose was confirmed independently using two differential histological staining methods employed to identify the location of lipid molecules, and lipid compartments. Thirdly, co-registration of histological and electrophysiological data was performed using intramural plunge needle mapping, which provided a stable electrode-myocardial interface. Assessment of ventricular tachycardia inducibility was confirmed on two separate occasions during the study. Moreover, activation mapping of the VT re-entrant circuits using plunge needle electrodes were complemented with global high-density non-contact 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 classical model has long been established as the cause of conduction slowing with the assumption that conduction traverses in a zig-zag pattern through
small myocardial channels within scar. Our data supports 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 classical model, we demonstrated that slowing of conduction, which is a pre-requisite for re-entry, is not exclusively altered by collagen.

Based on multiple recent clinical reports and case studies, the presence of intramyocardial adipose in patients with previous myocardial infarction may be underestimated. From those investigations, “lipomatous metaplasia” occurs in approximately 15-89% of patients with MI depending on the disease progression and modality of assessment used.\textsuperscript{1, 2, 11-13, 23} Based on those studies, histological assessment has greater sensitivity than MRI and CT-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.\textsuperscript{1, 2, 11-13} 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 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 visualisation of the electroanatomical maps.

Investigations from a large multicentre (MADIT II) trial revealed that in patients with ischemic left ventricular dysfunction, obesity was associated with an increased risk of ventricular arrhythmias resulting in appropriate device therapy, independent of diabetes.\textsuperscript{24} In contrast, we found that animal growth rate was not a significant factor in modulation of the VT substrate properties. All animals were fed the same diet, were of the same gender, 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 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. Electrical 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 Cx-43 proteins. However, observations from other studies have demonstrated that human pre-adipocytes are able to express other ion channels (I\textsubscript{to}, I\textsubscript{KCa}) in order to participate in cell proliferation.\textsuperscript{29} As such, adipose tissue, due to its high resistance and ionic membrane properties may act as a current sink, dampening the electrotonic interactions that occur between sparse neighbouring 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 45-85% above that observed for collagen-rich borders, based on increased Cx43 lateralization in adipose-bordering myocytes.

Whilst this data does 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. At a microscopic-scale, electrotonic coupling across collagen fibres between neighbouring myocytes has been observed.\textsuperscript{30,31} This mechanism of passive conduction may not necessarily reduce conduction velocities, 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 regarding the direct influence of adipose tissue on myocardium. One investigation demonstrated that adipokines, in particular, fatty acid binding protein 4 (FabP4), secreted by mature human adipocytes, were able to suppress contractility of rat cardiomyocytes.\textsuperscript{34} Whilst there have been no studies to examine 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 due to infiltration by adipose, can lead to altered mechano-electrical feedback in the tissue.\textsuperscript{35} Such altered mechano-electrical feedback is likely to manifest as ventricular ectopic beats early in the evolution of ventricular tachycardia circuits, and their persistence and frequency are likely to be involved in the ventricular remodelling process.\textsuperscript{35,36}

Induction of ventricular tachycardia was highly reproducible in this study spanning the continual remodelling process, indicating that the arrhythmogenic conditions were present within the early-healed phase of myocardial infarction. 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 due to 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 metallo-proteinase factors by the surrounding medium, which in turn degrades collagen.38, 39

It is well known that tissue structure, organisation, and function contribute to the dynamics of myocardial propagation. For re-entry to occur two conditions must be satisfied; (1) local conduction block, and (2) availability of sufficient time for the depolarising wavefront 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 wavefront has travelled during its refractory period.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 repolarisation time, smaller areas of block are sufficient to support re-entry. Based on our data, wavelength shortening was functionally dependent on conduction velocity, which was attributed to increased structural disarray. Our data strongly supports this mechanism of reentry, as excitation wavelength was significantly attenuated within substrate borders of animals with inducible VT, even after correction was performed for LV surface area. These VT anchor sites were in concordance with areas demonstrating earliest pre-systolic activity during VT, and exhibited a high degree of
adiposity.

Further research is warranted to determine whether intramyocardial adiposity is a by-product of pre-established reentrant circuits or whether there is sufficient adipogenic early remodelling within the arrhythmogenic substrate to consolidate early formed reentry circuits into the chronic stage of disease. One study of experimental myocardial infarction 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 at 24 to 48 hrs after occlusion. While this may contribute to long term remodelling, in our study, we were unable to demonstrate evidence of significant intracellular lipid accumulation in healed myocardial infarction using the current histological techniques.

Conclusions

The structural remodelling that occurs within the post infarct border zone differs between animals relative to the propensity of ventricular tachycardia. In this study, collagen was not a significant factor in determining the propensity to ventricular tachycardia induction. However, an increased intra-myocardial adipose density and the presence of narrow myocardial conducting channels were structural proponents that were significantly associated with critical re-entrant 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. As such, the formation and stability of these circuits were supported functionally by attenuation of excitation wavelength. Furthermore, the progressive remodelling 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 re-entrant circuits become established within the early healing phase, in light of characterising the phenotype, source, and paracrine effects of white adipose on myocyte function, and 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 intra-myocardial adipose within the infarct border zone using non-invasive techniques may be applied to predict propensity to post-MI VT and mortality. Moreover these findings can be used to develop novel therapeutic agents against cardiac adipogenesis.

Substrate mapping using 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 lipophylic compound, suggesting that high concentrations of the drug may accumulate within peri-infarct areas to promote pro-arrhythmia. Detailed clinical studies on the effect of anti-diabetic agents, which are capable of modulating adipogenesis, by selective stimulation of the peroxisome proliferator-activated receptor gamma (PPARγ) transcriptional pathway, may be warranted in patients post-MI.43-45

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

While 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, due to 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 to clinical manifestations of the disease, this post infarct ovine model has been well validated, and is representative of post infarct VT substrate in humans.\textsuperscript{16, 18, 46}

Serial assessment of myocardium was not performed in this study due to the limited robustness of available imaging modalities to identify intramyocardial adipose. However histological techniques employed in this study were able to quantify subtle differences in adipose volume using high resolution imaging and automated analysis to eliminate operator error. Although the histological data was derived from two dimensional sections, the data represents a three dimensional model of myocardium as the orientation of tissue excision, and orientation prior to sectioning were both randomised.

Acknowledgments: The authors are grateful to the staff of the Westmead Hospital Animal Research Facility for their assistance with the project and Virginia James (Westmead Millennium Institute, Sydney) for histological processing of specimens.

Funding Sources: This work was supported by a project grant from the National Health and Medical Research Council, Australia [grant #402669 to P.K.], a Sydney Medical School
Foundation Fellowship [E.K.] and a postgraduate scholarship from the Cardiac Society of Australia and New Zealand [J.P.].

Conflict of Interest Disclosures: None.

References:


Table 1.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Group</th>
<th>MI+VT-</th>
<th>MI+VT+</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (weeks) MI induction – EPS</td>
<td></td>
<td>7.2 (4-55)</td>
<td>7.8 (5-53)</td>
<td>0.974</td>
</tr>
<tr>
<td>EPS – Mapping Study</td>
<td></td>
<td>68 (2-124)</td>
<td>75 (4-160)</td>
<td>0.189</td>
</tr>
<tr>
<td>MI Induction</td>
<td></td>
<td>49 (45-55)</td>
<td>47 (37-55)</td>
<td>0.288</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPS Study</td>
<td></td>
<td>49 (46-55)</td>
<td>49 (37-56)</td>
<td>0.437</td>
</tr>
<tr>
<td>Mapping Study</td>
<td></td>
<td>57 (38-94)</td>
<td>58 (45-84)</td>
<td>0.761</td>
</tr>
<tr>
<td>Growth Rate (g/week)</td>
<td>MI induction – Mapping Study</td>
<td>200 (-83-538)</td>
<td>200 (72-293)</td>
<td>0.844</td>
</tr>
</tbody>
</table>

Between group comparison of animal growth characteristics, and time interval between studies. Data expressed as median (range). $p$ = probability based on comparison between MI+VT- and MI+VT+ groups.

Table 2.

<table>
<thead>
<tr>
<th>Electrophysiological Criteria</th>
<th>Histological Criteria</th>
<th>Viability</th>
<th>Contiguity</th>
<th>Collagen</th>
<th>Interstitial Adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>p</td>
<td>g</td>
<td>r</td>
</tr>
<tr>
<td>Amplitude</td>
<td></td>
<td>0.74±0.12</td>
<td>&lt;0.01</td>
<td>0.57</td>
<td>0.42±0.19</td>
</tr>
<tr>
<td>CV</td>
<td></td>
<td>0.47±0.21</td>
<td>0.03</td>
<td>0.16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$\lambda$</td>
<td></td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
<td>0.44±0.25</td>
</tr>
<tr>
<td>$\lambda$ (MI+VT-)</td>
<td></td>
<td>&lt;0.01</td>
<td>0.09</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>$\lambda$ (MI+VT+)</td>
<td></td>
<td>0.08</td>
<td>0.09</td>
<td>0.07</td>
<td>0.44±0.25</td>
</tr>
</tbody>
</table>

Correlations of histological and electrophysiological criteria. $r$ = mean within-sheep rank correlation (± s.d); $p$ = probability; $g$ = measure of effect magnitude.
Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Myocardial Viability</th>
<th>MI-</th>
<th>MI+ VT-</th>
<th>MI+ VT+</th>
<th>p</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>-</td>
<td>14.2±6.2</td>
<td>9.2±4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Below Average</td>
<td>-</td>
<td>9.0±6.5</td>
<td>5.1±4.5</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>3.9±2.7</td>
<td>0.549</td>
</tr>
<tr>
<td></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>dV/dt_{min}</td>
<td>Above Average</td>
<td>-</td>
<td>-0.23±0.18</td>
<td>-0.13±0.10</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Below Average</td>
<td>-</td>
<td>-0.12±0.08</td>
<td>-0.06±0.04</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>0.038</td>
<td></td>
</tr>
<tr>
<td></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>Local CV (m/s)</td>
<td>Above Average</td>
<td>-</td>
<td>0.83±0.28</td>
<td>0.64±0.35</td>
<td>0.084</td>
</tr>
<tr>
<td></td>
<td>Below Average</td>
<td>-</td>
<td>0.66±0.28</td>
<td>0.56±0.16</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>0.613</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>244±76</td>
<td>233±77</td>
<td>211±69*</td>
<td>0.247</td>
</tr>
<tr>
<td>\lambda</td>
<td>Above Average</td>
<td>-</td>
<td>267±85</td>
<td>199±100</td>
<td>0.455</td>
</tr>
<tr>
<td></td>
<td>Below Average</td>
<td>-</td>
<td>199±87</td>
<td>174±48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>169±54</td>
<td>169±47</td>
<td>0.161</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>265±73</td>
<td>197±54*</td>
<td>189±78*</td>
<td>0.258</td>
</tr>
<tr>
<td>\lambda_{Corrected}</td>
<td>Above Average</td>
<td>-</td>
<td>226±73</td>
<td>181±110</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Below Average</td>
<td>-</td>
<td>175±83</td>
<td>146±43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>150±50</td>
<td>132±50</td>
<td>0.407</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of electrophysiological criteria (mean ± sd) between groups with respect to viability. \( p \) = probability based on comparison between MI+VT- and MI+VT+ groups. * = \( p < 0.05 \) based on comparisons with MI- group.

Figure Legends:

**Figure 1.** Automated histological quantitation. A. Gomori-Trichrome stained paraffin section of myocardium excised from scar. B-C. Magnified section of epicardial and endocardial tissue demonstrating fibrous interlobular septa, characteristic of adipose tissue. D-E. Top right to bottom panels, demonstrating identification of myocardial structure using an automated algorithm. D. Total tissue area. E. Viable tissue area. F. Collagen area. G. Adipose area.
Figure 2. Examination of histological sections of fresh frozen myocardium excised from needle sites. The associated macroscopic and cellular structures of myocardium were confirmed using histochemical and immunohistochemical histological co-localization. Macroscopic section of A. dense scar, B. scar border, and C. normal myocardium. A2-A4. Magnification of A. indicating co-localization of myocytes, collagen, lipid laden adipocytes, and evidence of Cx43 expression at the scar interface. B2. Myocardium from within scar exhibiting mixed lateralized, and intercalated disk expression of Cx43 at myocyte junctions. C2. Myocardium, remote from the infarct zone, indicating normal cellular organisation, and expression of Cx43 at the intercalated disks of myocytes. GT= Gomori Trichrome; ORO = Oil-Red-O. Color index: Myocardium = red in GT, collagen = blue in GT, lipid laden adipocytes = red in ORO. Cx43 expression = red in Cx43+DAPI, Nucleli/Mitochondria = Blue in B2 and C2.

Figure 3. Microscopic examination of histological sections using light (A-B), confocal (C), and light-fluorescence microscopy (D-F). Gomori trichrome stained specimens of, A. Adipose from normal epicardium, and B. Adipose from scarred myocardium. C. Adipocytes from dense scar, indicating peripherally located nuclei (DAPI, blue) and absence of Cx43 expression. Example of sites expressing Cx43 at the intercalated discs of myocytes at variable distance from the scar interface; D. 800μm from Collagen; E. 1300μm from Adipose; F. 1650μm from mixed collagen and adipose. G. Quantitation of distance from scar interface, to zone where normal Cx43 expression at the intercalated disks of myocytes was observed relative to interface type. Color bar index associated with D. E and F. #=adipocytes.

Figure 4. Method used to calculate conduction velocity using multi-electrode plunge needles.
Left: Preferential pathway of conduction (□) recorded using multielectrode plunge needles. ◀ = Steepest gradient of conduction; • = interpolated grid; solid lines = isochrones; dashed circles = distance from central point at 2mm intervals. Right: Calculation of local conduction velocity at the central data point as a function of the slope of the linear regression line. \( d = \) distance from central data point; \( |\Delta t| \) = absolute difference in activation time along the preferential path of conduction from central data point.

Figure 5. Comparison of structural properties of myocardium between MI+VT- and MI+VT+ groups relative to myocardial viability. Collagen content did not differ between these groups, whereas significant impairment of myocardial contiguity and increased intramyocardial adipose was observed within the infarct border zone (areas exhibiting below-above average viability) of VT+ animals.

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.

Figure 7. Spatiotemporal relationship of VT re-entrant circuits to pathological features of the left ventricle post-MI. A. Example of global LV pre-systolic to late systolic propagation during VT in one animal using noncontact mapping. 3D Geometry orientation: projection=anterior, apex=inferior, septum=left. Corresponding 2D projections of inferior half of the LV chamber
are shown with superimposed isochrones. **B-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 due to limited access from the epicardium. **E-G.** Summary of collagen quantity, intramyocardial adipose quantity, and index of myocardial contiguity in all animals (MI+VT+), relative to VT activation time measured using intramural plunge needle electrode contact mapping. ★ = Earliest activation site.
Figure 1
Figure 2
Figure 3
Figure 4

Slope = Velocity

|Δt|
Figure 5
Figure 6
Figure 7

A. VT Activation Time (ms)

B. Scan (%) Contiguity Index

C. Interstitial Adipose (%)

D. VI Activation Time (ms)

E. ANOVA p=0.573

F. ANOVA p<0.001

G. ANOVA p=0.020

Collagen (%)

Interstitial Adipose (%)

Contiguity Index
Intramyocardial Adiposity Post-Myocardial Infarction: New Implications of a Substrate for Ventricular Tachycardia

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_Circulation_. published online September 13, 2013;
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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