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Assessment of coronary artery outward remodeling in consequence of excision of epicardial adipose tissue in Ossabaw swine

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Dedication

To mom and dad, I couldn't appreciate you more. Thank you for all the love, encouragement, and teaching me to work hard and try new things. You are truly the greatest people in the world.

To my siblings; Joanie, Chrissy, Bernie, Melanie, Angie and Kim- Taking on life with all of you has been the greatest adventure. Thank you for your unconditional love and filling my life with constant laughter. I could never thank you enough!

To my cousins, grandparents, friends, and Trevor- Thank you for the all of the words of encouragement and unwavering support. Life is better because of you all, and I am grateful for you always.

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To Dr. Lantzer, thank you for letting me know that quitting was not an option. I am thankful for your guidance and friendship throughout college.

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To Dr. Sturek and lab, thank you for welcoming me with open arms and teaching me so much in just one short day. I had a blast seeing what you all do!

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Abstract

Background
Coronary artery disease (CAD) results from the buildup of cholesterol, inflammatory factors, and proliferating smooth muscle cells within a vessel wall. This plaque impedes on the vessel lumen, decreasing the space through which blood can flow, leading to an array of complications in the human body. To offset these effects, the arterial wall undergoes outward remodeling, a compensatory physiologic phenomenon that blood vessels undertake when burdened with a blockage, such as CAD. In a previously conducted study, a coronary epicardial adipose tissue excision (cEATx) surgery was performed above the left anterior descending (LAD) in Ossabaw swine to investigate the effects of local adipose on the progression of CAD. Compared to the sham control group, pigs that underwent the adipectomy procedure revealed focal attenuation of disease progression at the surgical site within the LAD. Unlike, the previous research question, this current study aims to determine if there was an additional global outward remodeling effect by investigating disease progression in the right coronary artery (RCA) of the same animals. By comparing the two sites, we are able to determine whether the outward remodeling observed in the LAD was due to the local surgical procedure or a physiologic compensation for limitations caused by CAD progression.

Methods
Images of the RCA lumen were collected using intravascular ultrasound (IVUS). Measurements of the external elastic lamina and lumen area were taken of each collected still-frame image. For each pig, the data were averaged across the proximal 15 mm of the RCA at two separate time points (pre- and post-surgery). Pre-surgery measures were obtained the day the surgery took place while post-surgery measures were obtained 3 months later. Percent stenosis, plaque area, outward remodeling, and lumen area were all assessed.

Results
Progression of CAD in the RCA, represented by percent stenosis, was not significantly slowed in the adipectomy pigs compared to the control group. Outward remodeling in the RCA, represented by an increase in external elastic lamina circumference, was not significantly higher in the adipectomy pigs compared to the control group.

Conclusions
These data indicate that the cEATx procedure at the LAD did not attenuate CAD progression in the RCA.

Introduction

Obesity and metabolic syndrome in America

In the United States, over one-third of the adult population is obese (1). Obesity is defined as a body mass index (BMI) >30kg/m^2. Sedentary lifestyle, consumption of high-calorie foods, low socioeconomic status, and family history all increase a person's risk for becoming obese, This disease is becoming increasingly more prevalent because these risk factors are widespread among the American population. The manifestations of this disease negatively affect nearly every system in the body (1). Obesity, when measured as waist circumference, is one of five components of Metabolic Syndrome (MetS). MetS is defined by the National Heart, Lung, and Blood institute and the American Heart Association, as a condition that consists of three out of the five following risk factors: fasting plasma glucose ≥100 mg/dL, high density lipoprotein <40 mg/dL in men or <50 mg/dL in women, triglycerides 150 ≥ mg/dL, waist circumference ≥ 102 cm in men or ≥88 cm in women, and systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg (2).

Metabolic syndrome and coronary artery disease

MetS, or Syndrome X, has also been defined as a, "cluster of independent risk factors that increase the likelihood of cardiovascular disease" (3). Coronary Artery Disease, or CAD, is a disease process characterized by atherosclerotic plaque accumulation within the arteries that supply blood to the heart. The constellation of criteria for MetS creates an opportunity for CAD development due to the

atherogenicity of MetS. Atherosclerosis is a complex inflammatory condition that is characterized by plaque formation within blood vessels. The plaque-forming process begins with a fatty streak formation, and finally an atherosclerotic plaque forms (4). Fatty streaks are initial lesions caused by a local deposition of lipoproteins within the vessel's intimal layer (4). This lesion stimulates endothelial cells to then recruit inflammatory mediators and macrophages (4). As disease progresses further, foam cells arise within the lesion and contribute to the formation of plaque.

Lipoproteins that travel through blood vessels also play a role in atherosclerosis formation. Low Density Lipoprotein, or LDL is considered to be "bad cholesterol" because it contributes to the atherosclerotic plaque (5). In contrast, high-density lipoprotein (HDL) is found to be cardio-protective because it helps remove LDL from the arteries (5). Low HDL is a one out of the five components of MetS. According to the American Heart Association, The less HDL one has, the higher one's risk for heart disease (5). This point vividly illustrates the link between MetS and CAD.

Consequences of coronary artery disease

Without intervention, atherosclerotic plaques continue to grow and narrow on the vessel lumen, shrinking the area available for blood to flow and limiting perfusion to oxygen-dependent cardiac tissue. This decreased perfusion, or ischemia, lessens the oxygen supply to the heart muscles, which inhibits regular cell metabolism. If the plaque debris causes a blockage severe enough within the heart

or the brain, it could lead to a potentially fatal event, such as a myocardial infarction or stroke, respectively. Myocardial infarction, also known as heart attack, lessens the ability of the heart muscle to function properly, and therefore the heart cannot sufficiently deliver oxygen to the body. Every part of the body requires an oxygenated blood supply, which makes the manifestations of atherosclerosis extremely variable and potentially devastating. According to the American Heart Association, every 43 seconds, someone in the United States has a heart attack, and CAD is the leading cause (6). In later stages of CAD, plaques can calcify causing a decrease in vessel flexibility, further hindering its ability to pump blood.

Epicardial adipose tissue and coronary artery disease

Not only has obesity been correlated with cardiovascular risk, but more specifically, epicardial adipose tissue (EAT) volume expansion can occur independently of other risk factors for the disease (7,8). When the adipose tissue surrounding the coronary arteries releases chemical mediators such as cytokines and adipokines, it may encourage the formation of atherosclerotic plaque in an "outside-in" manner (8). Although EAT volume can independently increase cardiovascular risk, Dong, et al. observed that total EAT also positively correlates with other specific MetS risk factors, such as total cholesterol and low-density lipoprotein (9), which increases the risk of CAD. Furthermore, his study concluded that EAT volume was higher in the presence of plaques in patients with ≥50% coronary artery stenosis (9).

Outward remodeling

Due to the lumen-narrowing consequence of CAD progression, it has been observed that arteries attempt to adapt to resume normal functioning. Outward remodeling is a compensatory mechanism observed in human and non-human primates first described by Dr. Seymour Glagov in 1987 (10). It is an adaptation in patients who are suffering from focal stenosis in coronary arteries which increases the cross-sectional area of the vessel lumen in order to maintain adequate cardiac perfusion (Figure 1). Glagov explains that the rate of plaque accumulation and the outward remodeling response both independently vary between individuals (10).

In contrast to what has been observed clinically, Choy et al., with pigs as their

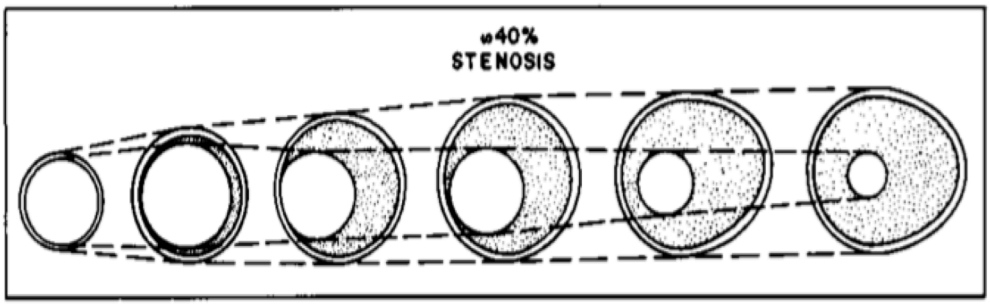

Figure 1. The Glagov phenomenon of outward remodeling (10). In early disease, the external elastic lamina expands outwardly to compensate for the encroaching plaque. After plaque has progressed to >40% stenosis, outward remodeling is no longer beneficial (10).

model, observed outward remodeling in diffuse CAD rather than only at focal stenotic lesions (11). This is important to note for the present study because Ossabaw swine classically develop diffuse, mild CAD rather than focal, severe lesions when fed an atherogenic diet for less than one year (12).

Intravascular ultrasound

The imaging modality used to collect data for this study was intravascular ultrasound (IVUS). IVUS produces live cross-sectional images of the inside of vessels

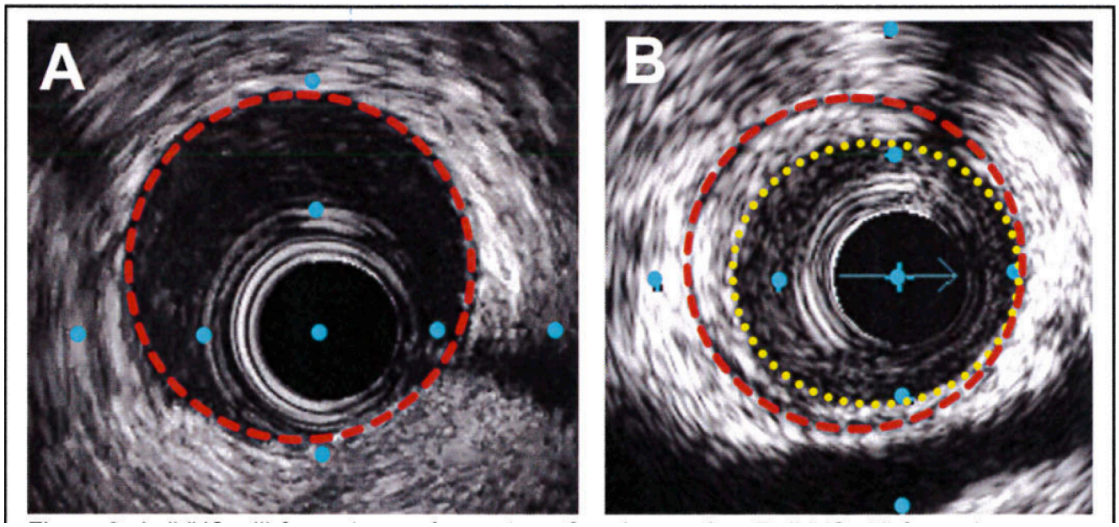

Figure 2. (A.) An IVUS still-frame image from a lean Ossabaw swine. (B.) IVUS still-frame image from an obese Ossabaw swine with MetS and CAD. (red dashed line = external elastic lamina; yellow dotted line = lumen; distance between blue dots = 1 mm)

(Figure 2). The IVUS subassembly is housed within a 1.6-mm plastic sheath to protect the arterial lumen from a spinning catheter. This includes a 20-MHz ultrasound transducer and a reflective mirror that deflects the ultrasound beam perpendicular to the long axis of the catheter and vessel. The distal end of the plastic sheath contains a radiopaque marker allowing for visualization of the IVUS catheter as it is fed through the femoral artery and up to the coronary arteries under fluoroscopic guidance and contrast-enhanced angiography (Figure 3). Once in place, the IVUS catheter is pulled back, distal to proximal, recording at a constant rate that produces 60 still-frames per millimeter.

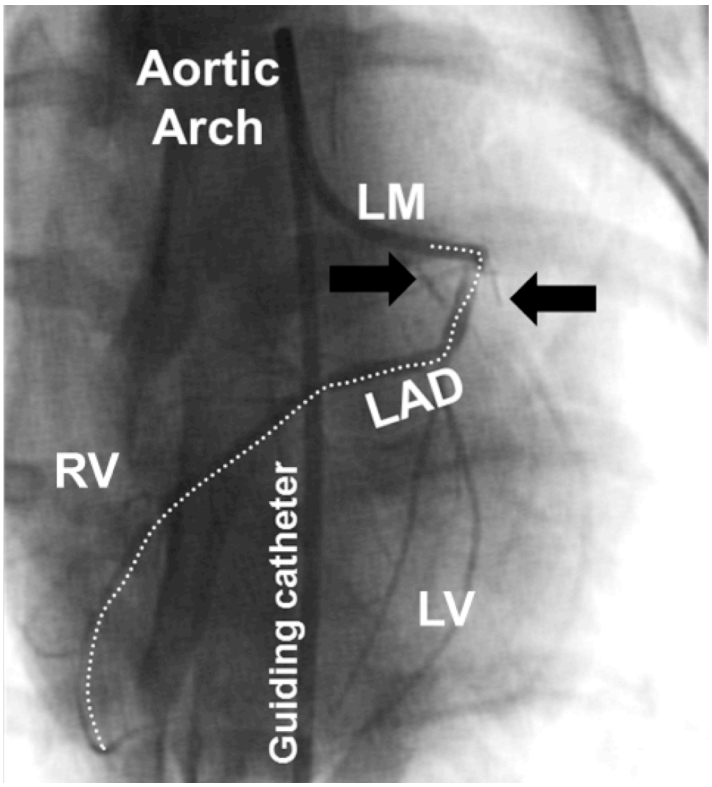

Figure 3. A contrast-enhanced AP angiogram of the Ossabaw heart. The imaging catheter is down the LAD (white dotted line). Black arrows indicate the proximal region of the LAD where data was collected for the original research study (19). The guiding catheter can be seen running up the thoracic aorta and over the aortic arch, ending at the left ostium at the LM. Image slightly modified from McKenney-Drake et al. (19). (LM = left main coronary artery; LV = left ventricle; RV = right ventricle)

There are many compelling reasons to use IVUS as an imaging modality of vessels. When used alone, contrast-enhanced angiography, has been questioned on its accuracy and reproducibility due to observer variability and its depiction of vessels as a planar silhouette (14). IVUS is more sensitive than angiography for demonstrating both the presence and the severity of atherosclerosis and calcification of the arteries (13). IVUS is favorable for visualization of arteries due to its ability to evaluate arterial wall thickness (13). With IVUS, one can also visualize the entire circumference of the vessel wall. The longitudinal nature of the imaging

also gives a unique view of the vessel and of the atherosclerotic plaque (14). The safety of IVUS is well documented. The most commonly reported adverse event is a transient spasm of the vessel, but this responds quickly to medication. The spasm may affect the quality and reliability of the image produced. There is always risk that the instruments of IVUS could damage a vessel, so it is important that the operator is trained and experienced in intracoronary catheter manipulation (14). There are, however, shortcomings to IVUS. The image produced is a two-dimensional image and it can be difficult to differentiate between thrombus, soft plaque, and hard plaque (15).

Ossabaw swine as a model of obesity, metabolic syndrome, and coronary artery disease

Human genetics allow us, as a species, to gain weight, and in some cases, become obese. Twin studies demonstrate that a predisposition for obesity is, up to 70% genetic (16). In his review, O'Rourke discusses the history of the human race to explain how humans become obese (16). In early years, humans lived an extremely active lifestyle with a limited food supply. Those individuals who were capable of storing calories in their adipose tissue during "feasting" times, and then able to burn those stored calories effectively in times of "famine" were most likely to survive. These "thrifty" metabolisms survived (15).

However, today, we are not living in a physically demanding world with limited resources. In the United States, it is quite the opposite. A typical American's lifestyle is sedentary with an abundance of food and resources that take virtually no energy to acquire, creating an "obesogenic" environment (16). This, in combination with the "thrifty" genotype we inherited from our ancestors, has led to an increase in obesity, MetS, and CAD. It is important to note that in humans CAD is

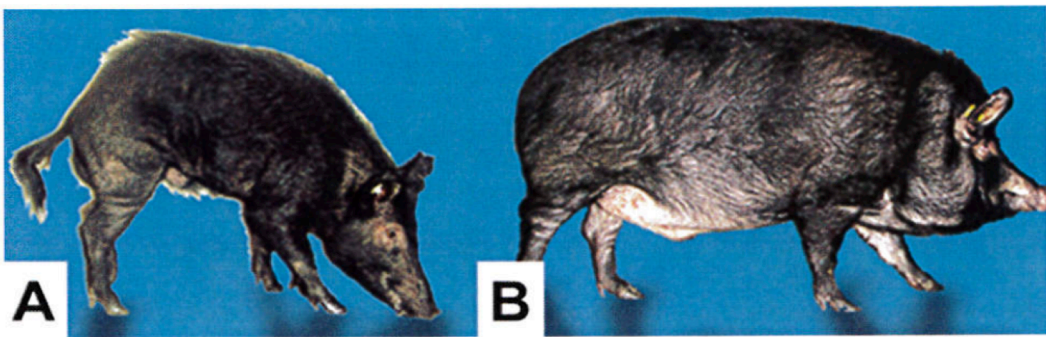

Figure 4. (A). Lean Ossabaw swine. (B.) Obese Ossabaw swine with MetS and CAD.

characterized by its symptom manifestation of atherosclerotic plaque buildup and lumen narrowing. However, the vessel occlusion in the Ossabaw swine was not significant enough to be symptomatic. For the purpose of this paper, CAD refers to the progression atherosclerosis in the Ossabaw swine's coronary arteries.

For biomedical research studies of obesity, MetS, and CAD, the Ossabaw miniature swine is an optimal animal model to use (Figure 4). Ossabaw have also developed a thrifty genotype due to the feast and famine cycle on their native Ossabaw Island off of the coast of Savannah, Georgia. Dr. Michael Sturek from the Indiana University School of Medicine and his team have validated the Ossabaw swine model by rigorously studying its similarities with human CAD progression in

the setting of obesity and MetS. When fed an excess-calorie atherogenic diet, sedentary Ossabaw swine naturally develop CAD and MetS, as opposed to other animal models that require genetic manipulations to induce disease (12,17). Furthermore, the hearts of pigs and humans are very anatomically and physiologically similar, making them an excellent model for conducting translational cardiovascular research.

Experimental adipectomy procedure and coronary artery disease progression in Ossabaw swine

In the previously conducted study, Ossabaw miniature swine with MetS were subjected to an adipectomy procedure in order to investigate whether or not the removal of coronary epicardial adipose tissue (cEAT) would alter the progression of CAD (19). In that study, female swine were randomized into two groups (sham and adipectomy) and fed the atherogenic diet for 8 months. For swine in the adipectomy group, cEAT was removed from proximal portion of the LAD (Figure 5) (19). Both adipectomy swine and others that underwent the sham surgery were then subjected to intravascular ultrasound (IVUS) in order to quantify the severity of CAD in all three coronary arteries.

For an additional 3 months, all swine were fed the same atherogenic diet prior to a secondary IVUS evaluation and euthanasia (19). This study specifically measured CAD changes in the proximal LAD. The data showed that the adipectomy, but not sham procedure halted CAD progression at the local surgical site and suggested plaque volume had regressed. Upon further investigation, the researchers

concluded that plaque size had not changed, rather the vessel had undergone positive outward remodeling. For the purpose of this thesis project, we investigated whether or not CAD was also halted in the right coronary artery (RCA) of pigs that underwent the adipectomy surgery.

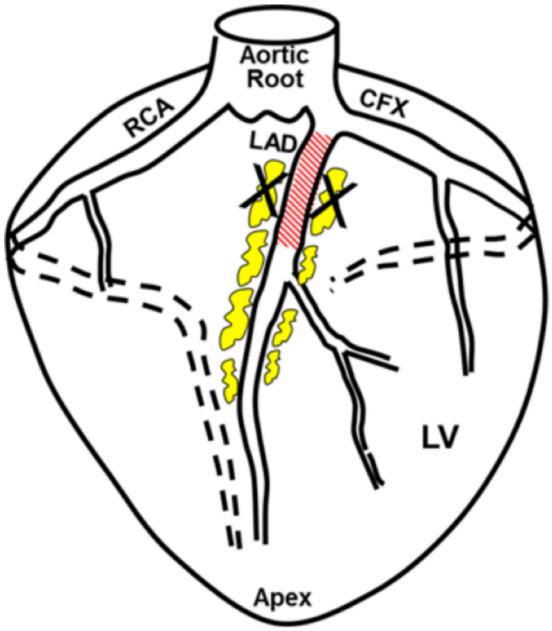

Figure 5. The adipectomy procedure was performed at the proximal LAD shaded in red. Data collection for this study was collected in the RCA. Slightly modified from McKenney-Drake et al. (19).

Methods

The original research study was approved by the Indiana University School of Medicine Animal Care and Use Committee (IACUC) and was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals.* After collecting the original RCA IVUS images, they were transferred to ImageJ software (National Institutes of Health) for analysis. This software arranges the images allowing for a longitudinal view of the vessel. The way the images are collected reflects the pull-back movement of the catheter and ultrasound probe from distal to proximal. Replicating the methods used in the original research study, quantification of CAD in the RCA was conducted within the proximal 15 mm of the vessel. In order to begin proximally, the right ostium must first be identified by its increase in lumen diameter just prior to entering the larger aorta. Once the initial still-frame at the right ostium was chosen, analysis occurred every 1 mm in vessel length, RCA IVUS data was collected and analyzed for each pig twice, once from the survival procedure (8 months on diet) and once from the sacrifice procedure (11 months on diet).

Measurements were conducted using the polygon tool within ImageJ. It is important to note that ultrasound waves create images based on the differences of densities of the anatomy around them. Therefore, IVUS cannot discern the internal elastic lamina (IEL), which separates the intima and the media layers of the coronary artery wall (Figure 6). In which case, the media layer is included in the calculation of percent stenosis when calculated the area of plaque burden. IVUS is able to identify the external elastic lamina (EEL), which surrounds the tunica media

of the vessel wall. For each still-frame image, the area of EEL was measured, as well

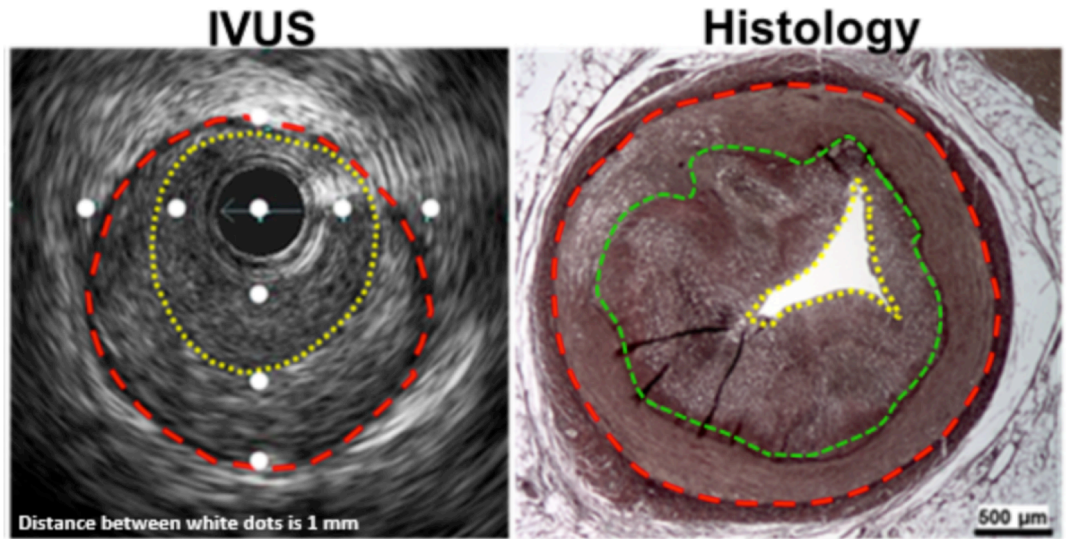

Figure 6. IVUS imaging demonstrates the EEL (red) and the lumen area (yellow). The IEL (green) is visible on histology, but not on IVUS. Slightly modified from McKenney-Drake et al. (18).

as the lumen area.

The collected data set was transferred into Microsoft excel for further analysis. All data points were averaged across the proximal 15 mm RCA of each pig for the survival and sacrifice procedures.

Initially, plaque area (PA) was determined using the following equation:

PA = EEL area – Lumen area

These values were used to calculate percent stenosis (PS) at each 1 mm interval using the following equation:

PS = (EEL area – Lumen area) / EEL area

Change in PS from survival to sacrifice procedures was then assessed by the following equation:

$$\mathbf{\Delta PS = PS_{sacrifice} - PS_{survival}}$$

Finally, outward remodeling, or a change in the EEL area from survival to sacrifice, was quantified by the following equation:

$$\mathbf{Outward\ Remodeling = EEL_{sacrifice} - EEL_{survival}}$$

All statistical analysis was performed and graphs were made with the software program, GraphPad Prism (GraphPad Software Inc., La Jolla, CA). Paired and unpaired Student's t-test were applied and statistical significance was set at $p<0.05$.

Results

Metabolic Data

As published in the original research study conducted by McKenney-Drake, et al. (19), there was no difference in body weights between the two pig groups throughout the study and at the time of euthanasia. Metabolic characteristics such as total cholesterol, triglycerides, fasting blood glucose, and blood pressures were also consistent between groups at the conclusion of the study.

Lumen Area

A two-tailed T test revealed that there was no significant change in proximal RCA lumen area in both sham (survival= 6.38±0.41 mm^2 vs. sacrifice= 6.99±0.45 mm^2, p=0.16) and cEATx groups (survival= 8.19 mm^2 vs. sacrifice= 7.53 mm^2, p=0.80;) (Figure 7).

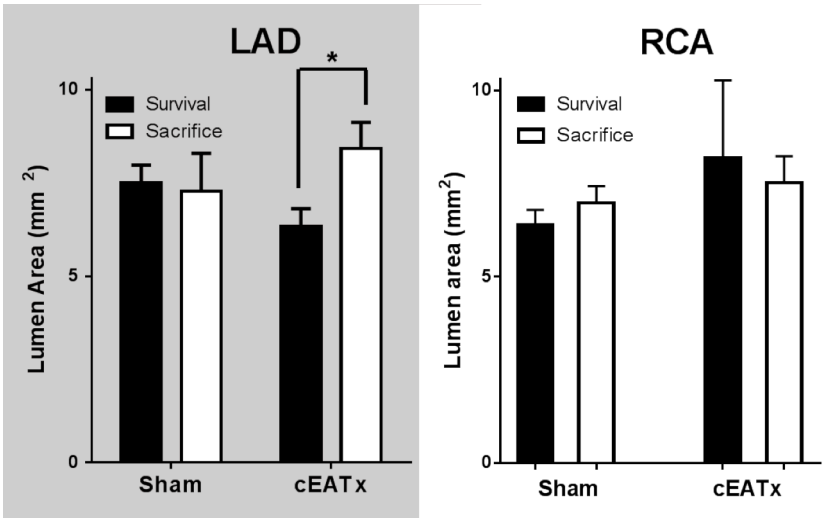

Figure 7. The lumen area within the proximal RCA did not change post-adipectomy procedure. The proximal LAD data from McKenney-Drake et al. (19) is supplied for reference (shaded in gray).

Plaque Area

A two-tailed t-test revealed that there was no significant change in plaque area within the proximal RCA in both sham (survival = 0.51±0.07 mm^2 vs. sacrifice= 0.61±0.17 mm^2 , p=0.63) and cEATx groups (survival= 0.70±0.25 mm^2 vs. sacrifice= 0.61±0.11 mm^2 , p=0.76) (Figure 8).

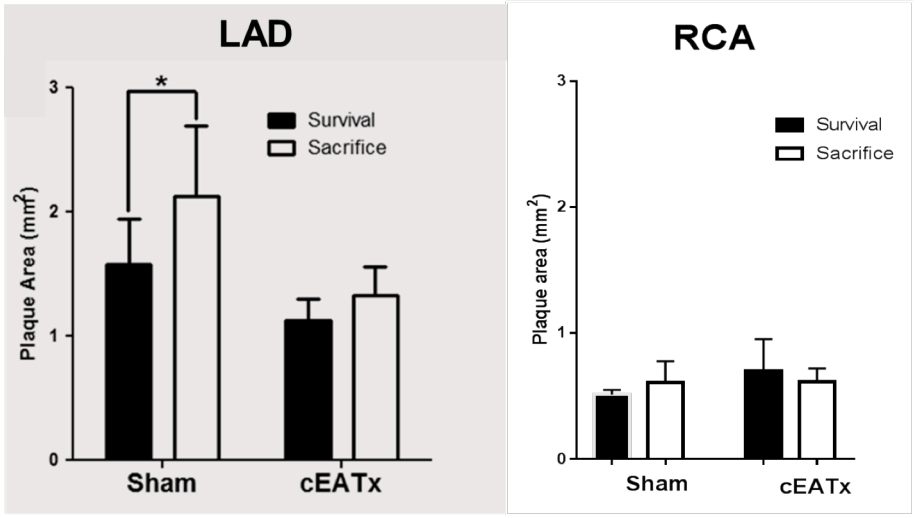

Figure 8. Proximal RCA plaque area did not significantly change in either sham or cEATx pigs. The proximal LAD data from McKenney-Drake et al. (19) is supplied for reference (shaded in gray).

Percent Stenosis

An unpaired two-tailed t-test revealed that the adipectomy procedure caused no significant change in percent stenosis within the proximal RCA between groups (Sham= 0.78±2.93 vs. cEATx= 0.08±1.22, p=0.79) (Figure 9).

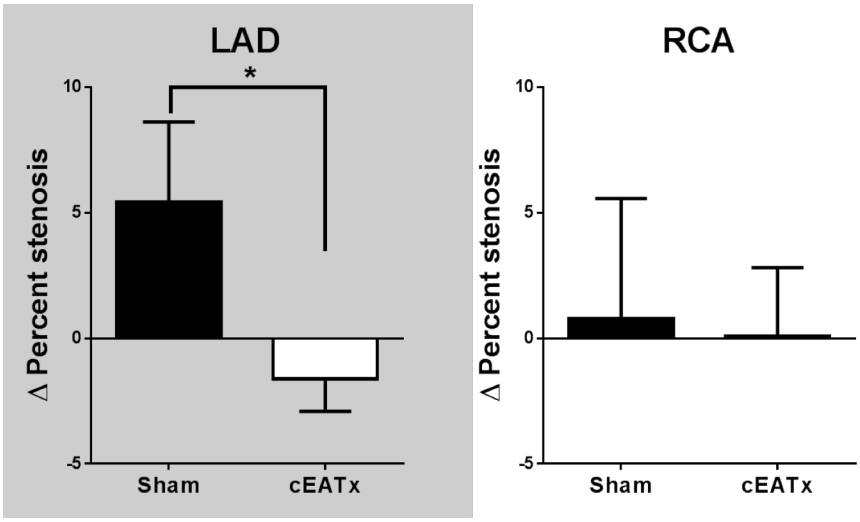

Figure 9. The adipectomy procedure caused no significant change in percent stenosis in the proximal RCA. The proximal LAD data from McKenney-Drake et al. (19) is supplied for reference (shaded in gray).

Outward Remodeling

An unpaired one-tailed t-test revealed that the adipectomy procedure did not cause outward remodeling within the proximal RCA (Sham= 0.71±0.24 mm^2 vs. cEATx= -0.84±2.61 mm^2, p=0.31) (Figure 10).

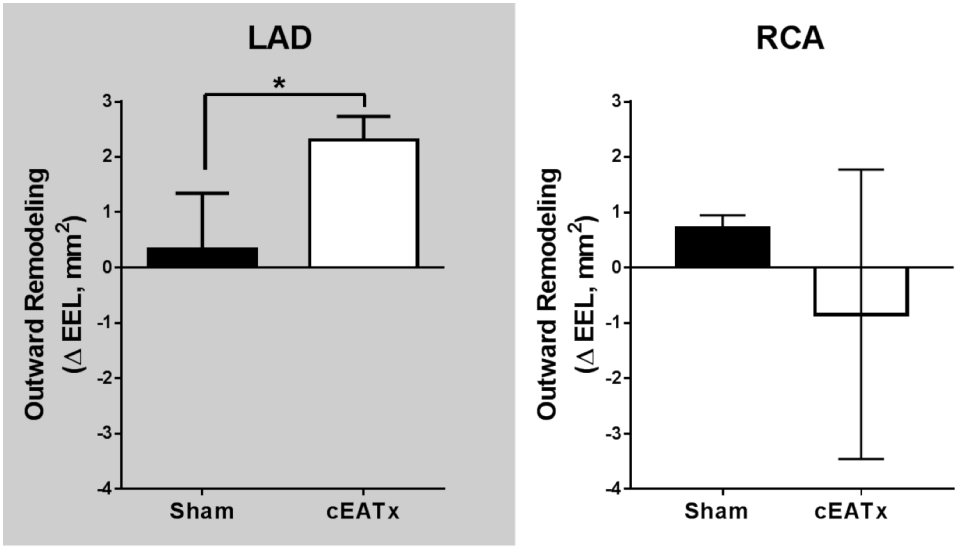

Figure 10. Outward remodeling was not observed in the proximal RCA of pigs who underwent the adipectomy procedure. The proximal LAD data from McKenney-Drake et al. (19) is supplied for reference (shaded in gray).

Discussion

The major finding of this study was that by performing an adipectomy procedure at the proximal LAD, the effects on CAD progression appears to be limited to the local LAD environment. When studied in the LAD, there was a significantly higher increase in percent stenosis and plaque area in the sham pigs compared to the cEATx group. Initially these data suggest plaque regression had occurred within the proximal LAD of cEATx pigs. However, one must also observe changes to both lumen and EEL in order to rule out any outward remodeling of the vessel wall. At sacrifice, the data in the LAD of CEATx pigs suggests outward remodeling had occurred (19). The data collected within the RCA suggested that the progression of CAD in the RCA was unaffected by the adipectomy procedure. In further development of the procedure and in future research on epicedial adipose tissue excision, it is important to note that the procedure had a local affect on CAD progression, as opposed to a global effect around the entirety of the coronary vasculature when looking at the RCA.

With the prevalence of cardiac disease in today's society, it is important to continue to explore options that can arrest atherogenesis or slow disease progression in the human vascular system. These developments can help people who are refractory to standard medications. Advancements such as this can contribute to the prevention of myocardial infarctions, cerebrovascular accidents, peripheral artery disease, and others. This does not suggest that adipectomy procedures should be performed in human patients, however, this research reinforces the role of cEAT volume in CAD.

There were some limitations in this study. The use of IVUS resulted in vasospasm in some of the arteries. . In this case, data cannot be used from those specific arteries. Furthermore, this shrunk the sample size of pigs. The small sample size reduces the internal validity of the data. Therefore, it is possible that if data were collected from a larger sample size, the results could have differed. Furthermore, the polygon tool on imageJ is prone to human error, so it is important to note that these measurements are just estimations and it is impossible to say that the measurements taken are completely accurate.

In further studies, the pigs could be injected with nitroglycerin or another vasodilator to reduce the risk of IVUS-induced coronary spasm. The timeline of the study between the adipectomy and euthanasia could also be increased to test the influence of the adipectomy on CAD over a longer time frame. It is important to take into consideration that lengthening the time frame would be a very expensive alteration to the study.

Conclusion

Excision of cEAT above of the proximal LAD of Ossabaw swine with MetS and CAD locally halted atherogenesis and increased outward remodeling. However, there was no effect on CAD progression in the RCA of the same pig suggesting the effects of an adipectomy procedure remain local to the surgical environment.

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