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Disconnect Between Adipose Tissue Inflammation and Cardiometabolic Dysfunction in Ossabaw Pigs

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Abstract

Objective—The Ossabaw pig is emerging as an attractive model of human cardiometabolic disease due to its size and susceptibility to atherosclerosis, among other characteristics. Here we investigated the relationship between adipose tissue inflammation and metabolic dysfunction in this model.

Methods—Young female Ossabaw pigs were fed a western-style high-fat diet (HFD) (n=4) or control low-fat diet (LFD) (n=4) for a period of 9 months and compared for cardiometabolic outcomes and adipose tissue inflammation.

Results—The HFD-fed “OBESE” pigs were 2.5 times heavier ($p < 0.001$) than LFD-fed “LEAN” pigs and developed severe obesity. HFD-feeding caused pronounced dyslipidemia, hypertension, insulin resistance (systemic and adipose) as well as induction of inflammatory genes, impairments in vasomotor reactivity to insulin and atherosclerosis in the coronary arteries. Remarkably, visceral, subcutaneous and perivascular adipose tissue inflammation (via FACS analysis and RT-PCR) was not increased in OBESE pigs, nor were circulating inflammatory cytokines.

Conclusions—These findings reveal a disconnect between adipose tissue inflammation and cardiometabolic dysfunction induced by western diet feeding in the Ossabaw pig model.

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Introduction

As obesity continues to increase, so does the prevalence of cardiometabolic diseases including coronary artery disease, stroke, peripheral vascular disease and type 2 diabetes. These disorders are major causes of overall morbidity and mortality in the U.S. and worldwide. Importantly, as obesity leads to cardiometabolic disease in some but not all cases⁽¹⁾, it is imperative that the mechanisms linking obesity to disease be better understood. Moreover, as obesity is now prevalent in children, it is becoming increasingly important to study the consequences of early life-onset obesity on cardiometabolic disease development⁽²⁾.

It is currently accepted that visceral obesity and insulin resistance (IR) form the ‘common soil’ from which cardiometabolic diseases develop, and that a central feature to this metabolic milieu is adipose tissue (AT) inflammation⁽³⁾. Visceral AT inflammation, including inflammatory macrophage (M ϕ) polarization, is predictive of metabolic dysfunction in several models with the majority of those conducted in rodents^(4, 5); relationships have also been observed between AT inflammation and metabolic dysfunction in humans⁽⁶⁾. Although research strides have been made to better understand such mechanisms, the vast majority of work has been done using rodents, whose size and rapid rate of maturation limits their ability to adequately model human obesity. Additionally, unlike humans, rodents do not develop atherosclerotic lesions unless genetically modified. The Ossabaw pig model is attractive because, similar to humans, when exposed to caloric excess and physical inactivity, they develop obesity and its metabolic consequences including IR, dyslipidemia, hypertension, and atherosclerosis⁽⁷⁾. The pig more closely resembles the human in terms of its size, growth rate, and development of cardiovascular disease and is emerging as a more appropriate obesity model⁽⁸⁾. The Ossabaw pig is characterized by the “thrifty phenotype” whereby this breed has adapted to store large amounts of energy during caloric excess⁽⁹⁾. Our group^(10, 11) and others^(12, 13) have been utilizing the Ossabaw as a model of cardiometabolic disease development. We previously demonstrated that significant metabolic changes, as well as AT⁽¹⁰⁾ and vascular⁽¹¹⁾ transcriptional alterations, occur early in the development of obesity in this model. Interestingly, the obesity that developed over that early period was not associated with increased expression of inflammatory genes conventionally viewed as being associated with obesity in visceral AT⁽¹⁰⁾ and coronary perivascular AT (PVAT)⁽¹¹⁾.

Although the Ossabaw is emerging as an important model of cardiometabolic dysfunction, the relationship between visceral AT inflammation and metabolic function in this model remains poorly understood. Here, we sought to extend our previous work in juvenile Ossabaw swine^(10, 11) and determine the effects of prolonged high fat diet (HFD) feeding through development and maturation (puberty around 5–6 month in swine) on AT

inflammation and cardiometabolic disease. We hypothesized that chronic HFD feeding of female Ossabaw pigs would result in significant cardiometabolic dysfunction in the absence of robust changes in AT inflammation owing to the thrifty phenotype and associated less harmful adipocyte expansion.

Methods

Animals, Diets, Blood Pressure, Body Composition, and Tissue Sampling

All procedures were approved by the ACUC at the University of Missouri. Juvenile (5–6 wk-old) female Ossabaw pigs (n=8) obtained from Michael Sturek, Ph.D. at the Ossabaw Swine Resource, Comparative Medicine Program at Purdue University and Indiana University School of Medicine were housed under temperature-controlled (20–23 °C) conditions with a 12 h/12 h light-dark cycle. Pigs were either limit-fed regular miniature pig chow diet (5L80, Lab Diet; 3.03 kcal/g; 10.5% fat, “LEAN” n=4) or a western HFD (5B4L, Lab Diet; 4.14 kcal/g; 40.8% fat and 17.8% high fructose corn syrup, “OBESE” n=4) for nine months. Since the Ossabaw swine will over-consume when given free food access, limit-feeding the LEAN as well as the OBESE group was necessary. Blood pressure was assessed by tail cuff method (GE Dash 3000) in conscious pigs after 8 ½ months of intervention and was the average of three measurements with at least a 10 minute separation between measurements. Following the intervention, pigs were weighed, body composition assessed via DXA (Hologic, QDR-1000), sedated by intramuscular injection of Telazol (5 mg/kg) and Xylazine (2.2 mg/kg) following an 18–20 hour fast, and blood collected for serum analyses via jugular vein. The pigs were then euthanized by IV injection of Telazol (10 mg/kg) and Xylazine (2.2 mg/kg) and removal of the heart. Subcutaneous abdominal (SQAT), visceral omental (OMAT), and PVAT surrounding the left anterior descending (LAD) coronary artery were harvested and processed or immediately frozen. The distal portion of the LAD coronary artery was dissected and used for vasomotor function experiments.

Serum and AT-Conditioned Media Analysis

Fasting serum glucose, NEFAs, total cholesterol, LDL, HDL, and TGs were analyzed as previously described (¹¹). Insulin was measured using commercial kits (Porcine Insulin ELISA, Mercodia, #10-1200-01). Serum and AT-conditioned media concentrations of interferon gamma (IFN- γ), interleukin (IL) 1 (IL-1 β), IL-1 receptor antagonist (IL-1RA), IL-6, IL-10, and tumor necrosis factor (TNF- α) were determined using a porcine-specific multiplex assay (Millipore Multiplex; Billerica, MA, #PCYTMAG-23K). All assays were run in duplicate.

Histology

SQAT, OMAT, and LAD coronary artery rings were fixed and stained with hematoxylin and eosin, as previously described (¹⁰). Digital images were captured with an Olympus BX60 light microscope and Olympus SC 100 camera (Waltham, MA). Adipocyte size was calculated based on 100 adipocytes/animal from six fields of view using Image J software as described previously (¹⁴). Separate slides were stained with porcine-specific anti-scavenger

receptor class A (SRA) (Anti MSR-A/CD204, 1:100, Cosmo Bio USA, #KAL-KT022) antibody and examined by an investigator blinded to the treatment groups.

Fluorescence Associated Cell Sorting (FACS)

The stromal vascular cell (SVC) fraction was isolated from whole AT extracted from SQAT, OMAT, and PVAT depots via collagenase digestion as previously described⁽¹⁴⁾ with slight modifications. The following porcine-specific fluorophore-conjugated antibodies were used: CD3ε-PerCP-Cy5.5 (BD Pharmingen, #561478), CD4a- PEcy7 (BD Pharmingen, #561473), CD8a-Alexa Fluor 647 (BD Pharmingen, #561475), and CD68- FITC (Santa Cruz Biotechnology, # sc-7083 FITC). Gating strategies included dead cell discrimination and lymphocyte quantification based on forward/side scatter and included unstained cells, single stain, and FMO controls. Cells were immunophenotyped using a CyAN ADP Analyzer (Beckman Coulter, Inc.) and data analyzed using Summit 5.2 (Beckman Coulter).

RNA Extraction and Quantitative Real-time RT-PCR

Quantitative real-time PCR was performed as previously described⁽¹¹⁾ and reactions were performed in duplicate. Primer sequences are available upon request. 18S was used as house-keeping control gene and cycle thresholds (CT) were not different between groups across for any tissues. mRNA expression values are presented as 2^{-CT} whereby $CT = 18S$ $CT -$ gene of interest CT and normalized to LEAN, set at 1.

Cytokine Secretion from AT

A portion of SQAT, OMAT, and PVAT surrounding the LAD coronary artery were incubated in Medium 199 (pH 7.4, 24 hrs) (100 mg AT /500 µl) under standard culture conditions (37°C, 5% CO₂) as described⁽¹⁵⁾ to produce AT-conditioned media.

Vasomotor Function Experiments in LAD Coronary Artery Rings

Distal end of the LAD coronary arteries were exposed from the heart and microdissected in the chamber at 4°C. Coronary ring segments were cut into 2-mm rings and mounted in a myograph chamber (Model 610M, Danish Myo Technology, Aarhus, Denmark) containing physiological salt solution gassed with 95% O₂-5% CO₂ at 37°C, as previously described⁽¹¹⁾. After a 30-minute equilibration period, an optimal tension (25mN) was applied and then another 30 minutes of equilibration followed. Rings were stimulated with cumulative addition of K⁺ (30–120 mM) to assess vessel viability. Coronary rings were precontracted with 10nM U-46619 to induce ~70–80% maximal contraction (i.e. relative to maximal U-46619-induced contraction; data not shown). Concentration-response curves were obtained by cumulative addition of either bradykinin (10⁻¹² to 10⁻⁷M), insulin (1 to 1000 µIU/mL) or sodium nitroprusside (10⁻⁹ to 10⁻⁵M). Relaxation at each concentration was measured and expressed as percent maximum relaxation, where 100% is equivalent to loss of all tension developed in response to U-46619.

Statistical Analysis

Between group differences were determined using Student's two-tailed t-tests and considered statistically significant if $P < 0.05$. Statistical analysis was performed using SPSS 22.0; all data are presented as mean \pm SEM.

Results

HFD Induces Obesity, IR, and Dyslipidemia

The average body weight for the Ossabaw swine before randomization was 4.9 ± 0.3 kg. Throughout the study, LEAN pigs were limit-fed to an average of 600 g food/day; whereas the OBESE pigs were limit-fed to an average of 1200 g/day. Compared to LEAN, OBESE were ~2.5X heavier and had 45% more body fat (Table 1). OBESE were considerably larger animals, indicated not only by greater adiposity but also by greater length, lean, bone, and heart mass (Table 1). Adipocytes from OMAT were more than twice as large in the OBESE than in the LEAN group ($p < 0.001$, Figure 1). However, adipocytes from SQAT were not different in size between groups ($p = 0.32$, Figure 1). Compared to LEAN, OBESE also had higher fasting total cholesterol, HDL, LDL, NEFAs and TGs (all $P < 0.001$, Table 1). OBESE were also considered diabetic based on elevated fasting plasma glucose and were significantly more insulin resistant based on the homeostatic model assessment (HOMA-IR⁽¹⁶⁾) and adipocyte IR (Adipo-IR⁽¹⁷⁾) (Table 1). Confirming what others have previously reported⁽¹²⁾, despite only obtaining blood pressure measurements on a single day, OBESE also had significantly elevated systolic and diastolic blood pressure compared to LEAN.

Markers of Inflammation Not Increased in Circulation, AT, or AT-Conditioned Media of OBESE

Of the circulating cytokines measured (IL-10, TNF- α , IFN- γ , IL-1 β , IL-1RA, IL-6), only IL-6 was different between OBESE and LEAN with OBESE having ~60% lower circulating values (0.0425 ± 0.006 (LEAN) vs. 0.0165 ± 0.003 (OBESE) pg/mL, $P = 0.012$) (Supplementary Figure 1). When media conditioned with AT from LEAN and OBESE was assessed for cytokines (i.e., as indication of AT cytokine production), no between-group differences were observed in any of the cytokines measured. Similarly, no differences in AT immune cell infiltration were observed between OBESE and LEAN pigs. From the SVC fraction, CD68+SVCs (M ϕ s) and CD3+, CD3/4+, CD3/8+SVCs (T lymphocytes) were isolated from AT harvested from OMAT, SQAT, and PVAT and quantified via FACS. In concordance with the lack of systemic inflammation in the OBESE, we did not detect increased AT T lymphocytes or M ϕ s in the OBESE compared to the LEAN in any of the depots. Finally, in accordance with the lack of evidence of AT inflammatory cell infiltration, it did not appear that OBESE OMAT or SQAT displayed increased M ϕ content as measured via SRA (M ϕ marker) immunostaining (Supp. Fig. 1).

Little Evidence of AT Inflammation in OBESE Via Gene Expression

To further examine the inflammatory profile of AT from OBESE and LEAN, a comprehensive gene expression panel was analyzed in OMAT and SQAT (Figure 2). In OMAT, only five genes were significantly up-regulated in OBESE pigs. Adiponectin, an AT-

secreted protein known to be insulin sensitizing and anti-inflammatory, was elevated ~2-fold and leptin, another AT-secreted protein important in metabolic homeostasis, was ~7-fold higher. IL-6, a cytokine secreted by immune cells as well as adipocytes that is thought to be “immunomodulatory” was ~3-fold higher in OMAT from OBESE animals. No other inflammatory markers were elevated (TNF- α , IFN- γ , toll-like receptor (TLR4), inflammatory T cell markers) except for monocyte chemoattractant protein (MCP-1), important in drawing in M ϕ s, which was ~4-fold elevated in OBESE. The T helper cell marker, CD4, trended to be higher among OBESE ($P=0.076$). Interestingly, the naturally occurring anti-oxidant molecule, superoxide dismutase (SOD1) was also marginally elevated in OBESE OMAT as was PPAR λ ($P=0.08$), a nuclear receptor known to enhance adipocyte insulin sensitivity and reduce inflammation (¹⁸) (Figure 2A). No markers of M ϕ infiltration were elevated in OBESE compared to LEAN OMAT, while the M ϕ markers CD14 and CD16 were marginally suppressed in OBESE OMAT.

In SQAT, two genes were significantly higher in OBESE: CYBB (GP91-phox), an NADPH oxidase subunit indicative of oxidative stress, and the alternative/anti-inflammatory M ϕ marker known to produce anti-inflammatory cytokines, CD163 (Figure 2B). In stark contrast to other animal models of obesity, gene expression of CD4 (indicative of T helper cells) and CD8 (indicative of cytotoxic T cells) were lower in OBESE compared to LEAN as was the pro-inflammatory cytokine, IFN γ , which is indicative of inflammatory T cell activation (¹⁹) and the M ϕ marker, CD16. Two markers indicative of T regulatory cell (Treg) activation, Foxp3 and CTLA4, were not suppressed in OBESE, however. Tregs have been shown to have anti-inflammatory and insulin-sensitizing properties in AT (²⁰). However, no differences were observed in CD3, CD4, or CD8+ T cells via FACS in SQAT between groups. These findings indicate that the OBESE pigs studied here did not experience increased SQAT T cell and/or M ϕ influx.

OBESE Have Impaired Insulin-Stimulated Vasorelaxation and Atherosclerotic Lesion Formation in LAD Coronary Arteries Despite no Increase in PVAT Inflammation

Upon histological examination, the OBESE pigs exhibited early evidence of atherosclerotic lesion formation in the LAD coronary arteries. Specifically, as shown in a representative 40x H&E-stained image, we observed foam cell formation in the subendothelial space and intima-medial thickening of the artery wall (Figure 3A, top panels). In addition, we noted positive SRA staining on the luminal surface of LAD coronary arteries from OBESE pigs, indicative of greater inflammatory M ϕ s (Figure 3A, bottom panels). These are considered early-stage lesions based on the histological classification of atherosclerosis published by the American Heart Association Committee on Vascular Lesions. Similarly, several inflammatory genes were, or trended toward being, up-regulated in the LAD coronary artery of OBESE vs. LEAN including the chemokines, MCP-1 ($P=0.057$), VCAM1 ($P=0.11$), and ICAM ($P=0.18$), the M ϕ marker, F4/80 ($P=0.15$), and NADPH oxidase subunits, p47Phox ($P=0.067$) and p91Phox ($P<0.05$) (Figure 3B). We also measured expression of the same genes in PVAT adjacent to the LAD coronary artery. Similar to the lack of inflammation detected in other depots, the PVAT of the OBESE did not express higher inflammatory gene expression (Figure 3C). No genes were significantly different between OBESE and LEAN with the exception of CD3 ($P<0.05$), CD8 (trending at $P=0.09$), and IFN- γ ($P<0.05$), which

all were down-regulated in OBESE. As shown in Figure 4, insulin-stimulated relaxation, but not bradykinin or sodium nitroprusside-induced relaxation, in the LAD coronary artery was blunted in OBESE compared to LEAN.

Discussion

We previously demonstrated that juvenile HFD-fed Ossabaw swine develop obesity and IR, with minimal evidence of AT inflammation⁽¹⁰⁾. Here, we extend our previous work, demonstrating that, despite the fact that continued overconsumption of HFD into early adulthood causes extreme obesity, dyslipidemia, systemic IR, vascular IR, hypertension, as well as coronary artery inflammation and atherosclerotic lesions, female Ossabaw swine remained largely “protected” from the development of AT and systemic inflammation traditionally viewed as “characteristic” of obesity-associated metabolic impairments.

The HFD-fed OBESE pigs did not exhibit increased visceral AT (i.e., OMAT) M ϕ or T cell infiltration assessed by FACS and verified at the level of mRNA in several inflammatory markers including T cell markers (CD3, CD8) and M ϕ markers (CD68, CD14, CD16). The OBESE OMAT expressed higher levels of adiponectin and leptin, which often associate with greater adipocyte size, but the vast majority of inflammatory M ϕ , T cell, and cytokine markers were not increased. However, the chemoattractant, MCP-1, thought to precede immune cell infiltration into AT, was significantly increased in OBESE AT. This is consistent with other findings in this model in the absence of significant inflammatory M ϕ infiltration⁽¹²⁾, and may suggest that MCP-1 is recruiting anti-inflammatory rather than inflammatory M ϕ s since we observed an increase in the alternative M ϕ marker, CD163, in OBESE SQAT. Gene expression of the nuclear receptor, PPAR λ trended higher in OBESE OMAT ($P=0.08$) and SQAT ($P=0.12$). PPAR λ associates with greater insulin sensitivity, less dysregulation of adipocyte lipolysis and an anti-inflammatory M ϕ profile⁽¹⁸⁾. Also interesting, SOD1 (an antioxidant) was significantly elevated in OBESE OMAT.

Cytokine release from AT explants harvested from the three depots investigated (i.e., SQAT, OMAT, and PVAT) did not differ between groups, nor were there increases in circulating inflammatory cytokines (TNF- α , IFN- γ , IL-1 β). Intriguingly, despite increased IL-6 gene expression in OMAT in OBESE pigs, OMAT secretion was unaltered and circulating levels were significantly reduced. This may suggest a disconnect between transcription and translation. IL-6 has been shown to be increased in AT⁽²¹⁾ and circulation of obese humans⁽²²⁾ and correlate both inversely⁽²³⁾ and positively⁽²²⁾ with IR. IL-6 has both AT and skeletal muscle origins⁽²⁴⁾, and considered by some to be both pro- and anti-inflammatory⁽²⁵⁾. It is possible that reduced skeletal muscle IL-6 may have contributed to reduced circulating levels in the OBESE pigs. This possibility should be addressed in future studies.

Taken together, Ossabaw swine appear to be protected from HFD-induced increases in AT inflammation. These findings correspond with our previous work in juvenile Ossabaw swine fed a HFD shorter-term⁽¹⁰⁾ and another previous report where HFD-feeding (~ 7 months) failed to increase CD203+ M ϕ infiltration (i.e., less CD203+SVCs isolated from AT of HFD-fed vs control pigs)⁽¹²⁾. In that study, CD203 was used as a marker of mature M ϕ s similar to the marker we used to identify cells of the M ϕ /monocyte lineage, CD68; both are

non-specific M ϕ markers. Interestingly, although HFD reduced total AT M ϕ infiltration in the Faris study, it caused the M ϕ phenotype to change such that a greater percentage expressed CD16, a marker thought to be associated with inflammatory M ϕ activity (12).

The SQAT is generally considered a healthier AT depot and is characterized by smaller, more insulin sensitive and less inflammatory adipocytes (26). Still, adipocytes in this depot have been shown to expand with obesity in other models, albeit not to the extent to which adipocytes from the visceral region do (6). Reduced expandability of adipocytes from SQAT during the progression of obesity may potentiate ectopic lipid deposition and increase visceral adiposity, all of which contribute to IR (27). The “adipose tissue expandability” hypothesis is that when adipocytes are limited in their ability to expand, this results in adipocyte stress, inflammation and IR (28). The lack of expandability of SQAT adipocytes in the OBESE may have contributed to their larger OMAT adipocytes as well as the increase in systemic IR and dyslipidemia. Our data suggest that this animal model, compared to others, is protected to some degree in terms of AT inflammation and that less harmful adipocyte ‘expandability’ may be contributing to this protection. Importantly, AT inflammation is not always present in obese adults (29, 30) and it recently has been reported that overweight children showed little evidence for M ϕ s in AT (31). Thus, the lower susceptibility to AT inflammation in the pig may lend support for the use of pig (as opposed to rodent) models to more accurately parallel the metabolic manifestations of obesity in humans.

Given the lack of overt AT inflammation, which is the major source of obesity-associated systemic inflammation (32), it was not surprising that OBESE did not have greater systemic inflammation. However, the OBESE pigs developed other features of cardiometabolic dysfunction including hypertension, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and impaired insulin-stimulated vasodilation in coronary arteries. The OBESE also developed atherosclerotic lesions and increased coronary artery inflammation and oxidative stress. Indeed, the Ossabaw has been described as one of the best porcine models of metabolic syndrome-induced atherosclerosis (33). The role played by PVAT in the pathophysiology of cardiovascular disease is becoming increasingly appreciated with evidence suggesting that inflammatory factors secreted by PVAT promote inflammation and impair vascular function (34, 35). Remarkably, although significant up-regulation of genes associated with inflammation were detected in the LAD, such genes were not elevated, and many reduced, in PVAT. These findings are consistent with recent data showing that most of the PVAT secreted proteins that were altered with obesity in Ossabaw were not related to classic markers of inflammation or oxidative stress (35). Similarly, our previous microarray analysis in coronary PVAT from juvenile lean and obese Ossabaw revealed only 7 genes were significantly altered with obesity (11), none of which were linked to inflammation or oxidative stress pathways. Together, these findings point to a disconnect between AT inflammation and cardiometabolic dysfunction in the Ossabaw model. An important question is whether this disconnect is specific to the Ossabaw or is consistent across swine breeds. Compared to other models, the Ossabaw is arguably the best model of metabolic syndrome-associated cardiometabolic dysfunction for a variety of reasons including practicality of their smaller size and development of human cardiometabolic manifestations. However, the limited data available in other breeds suggests that pigs in general may not be as susceptible to metabolic inflammation. Female HFD-fed White pigs develop some

evidence of systemic inflammation, but no increase in IL-6 or AT Mφs⁽³⁶⁾; Gottingen pigs⁽³⁷⁾ and a swine model of familial hypercholesterolaemia⁽³⁸⁾ also appear protected against systemic inflammation. While insufficient data are available to make conclusions regarding the breed-specificity of the lack of AT inflammation documented in our study, the available evidence suggest that the pig model is less susceptible to obesity-induced AT inflammation compared to other models. Another important consideration is that there are known sex differences in obesity-induced AT inflammation such that ovary-intact females are less susceptible to AT inflammation compared to age-matched males; whether this protection would be seen in male Ossabaw pigs is unknown. Unfortunately, the other cited studies^(12, 37) investigating metabolic inflammation in pigs were also exclusively in females.

Insulin-stimulated relaxation in the LAD coronary artery was blunted in OBESE versus LEAN pigs in the absence of changes in bradykinin-induced relaxation, a response largely endothelium-dependent. In line with this observation, compelling evidence from studies using obese rodents demonstrate that impairments in insulin-stimulated dilation occur prior to impairments in other endothelium-dependent dilators in both skeletal muscle and coronary arteries^(39, 40). Reciprocally, our group found that an improvement in insulin-induced dilation with physical activity-induced weight loss occurs in the absence of changes in acetylcholine-mediated dilation in rats⁽¹⁵⁾. Thus, it appears that obesity-related changes in vascular insulin sensitivity do not always correlate with changes in classic measures of endothelium-dependent dilation.

Remarkably, after nine months of HFD feeding, the Ossabaw pigs studied here were largely “protected” from AT and systemic inflammation despite developing severe obesity with visceral adipocyte size expansion, IR, atherosclerosis, and dyslipidemia. These findings suggest that visceral AT inflammation is not a “hallmark feature” of the development of cardiometabolic disease in the female Ossabaw pig model. Given that AT inflammation has been shown to predict adverse metabolic outcomes in humans and rodents⁽⁶⁾, determining what factor(s) is “protecting” the Ossabaw from developing inflammation could lead to therapeutic or preventative strategies applicable to human cardiometabolic disease. We speculate that the Ossabaw pig, and perhaps other swine breeds, have evolved to survive despite gross AT expansion owing to their “thrifty” genotype and postulate that this genetically-selected less harmful AT expansion may buffer the Ossabaw pig from a further exacerbation in cardiometabolic pathologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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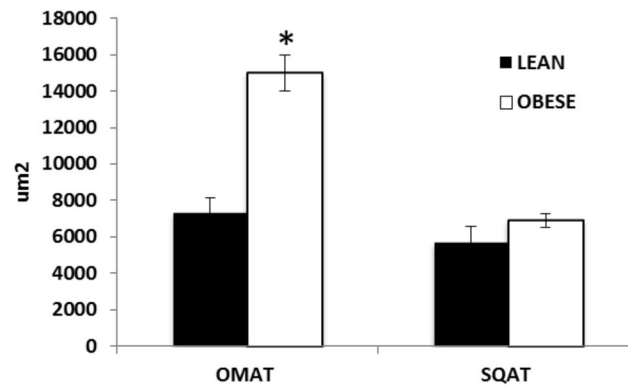
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- The Ossabaw swine model is emerging as an appropriate model for human cardiometabolic disease, but the role of adipose tissue inflammation on the development of cardiometabolic disease in this model has not been extensively investigated. As such, this study's objective was to investigate the relationship between adipose tissue inflammation and cardiometabolic disease in Ossabaw swine.
- Findings demonstrate that feeding Ossabaw swine a western high fat diet, from an early age into adulthood, results in severe obesity and cardiometabolic impairments without increased adipose tissue inflammation.
- Future study of the adipose tissue metabolic phenotype of the Ossabaw pig model may lend important insight into the relationship(s) between adipose tissue immunometabolism and human diseases.

A. Adipocyte Mean Cell Size



B. Representative Images

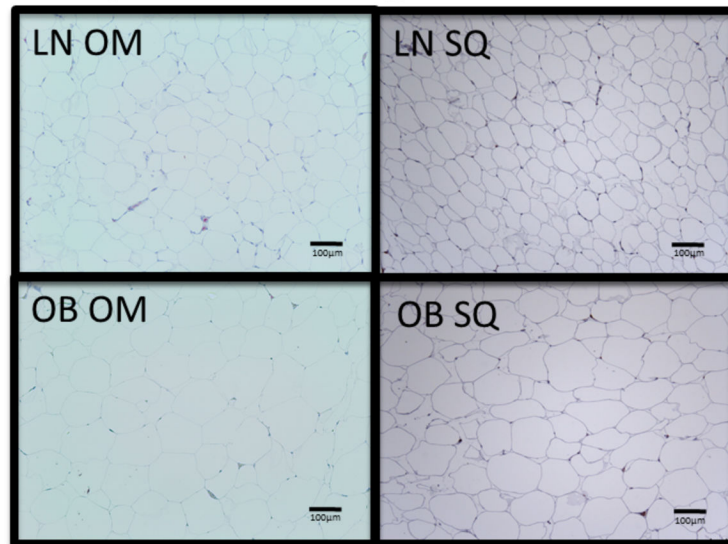


Figure 1. OBESE pigs have greater mean adipocyte cell size in omental visceral AT (OMAT), but adipocytes from subcutaneous AT (SQAT) were not different between LEAN and OBESE
 A: Mean adipocyte cell size calculated based on counts for 100 adipocytes/animal; n=4 animals/group. B: Representative images for each depot. Data expressed as mean \pm SEM; * P<0.05; LN = LEAN; OB=OBESE; OM = OMAT; SQ = SQAT.

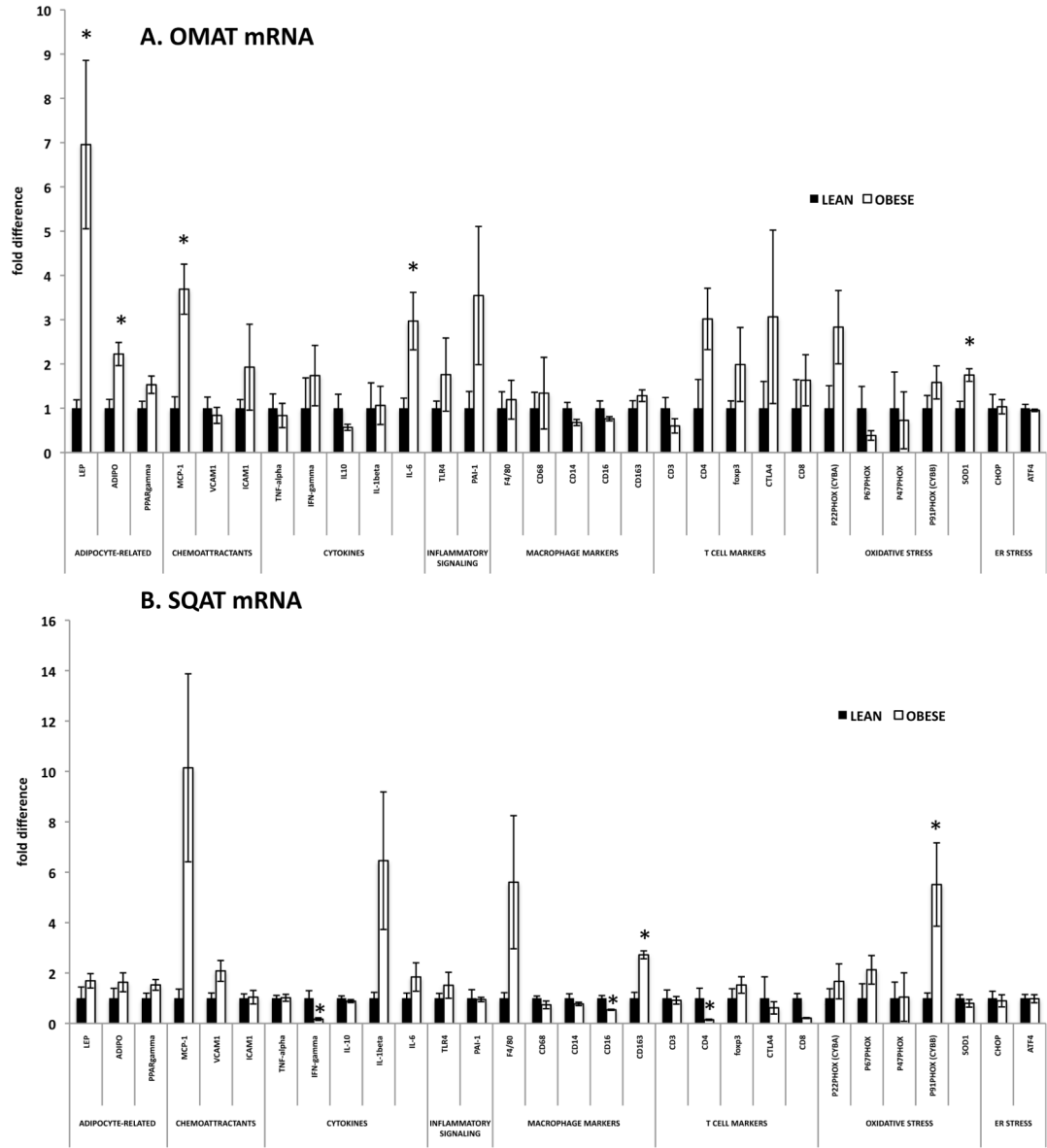


Figure 2. Little change in AT inflammatory gene expression in OBESE pigs
 A: Omental AT (OMAT) gene expression. B: Subcutaneous AT (SQAT) gene expression.
 Data expressed as mean \pm SEM; * P<0.05

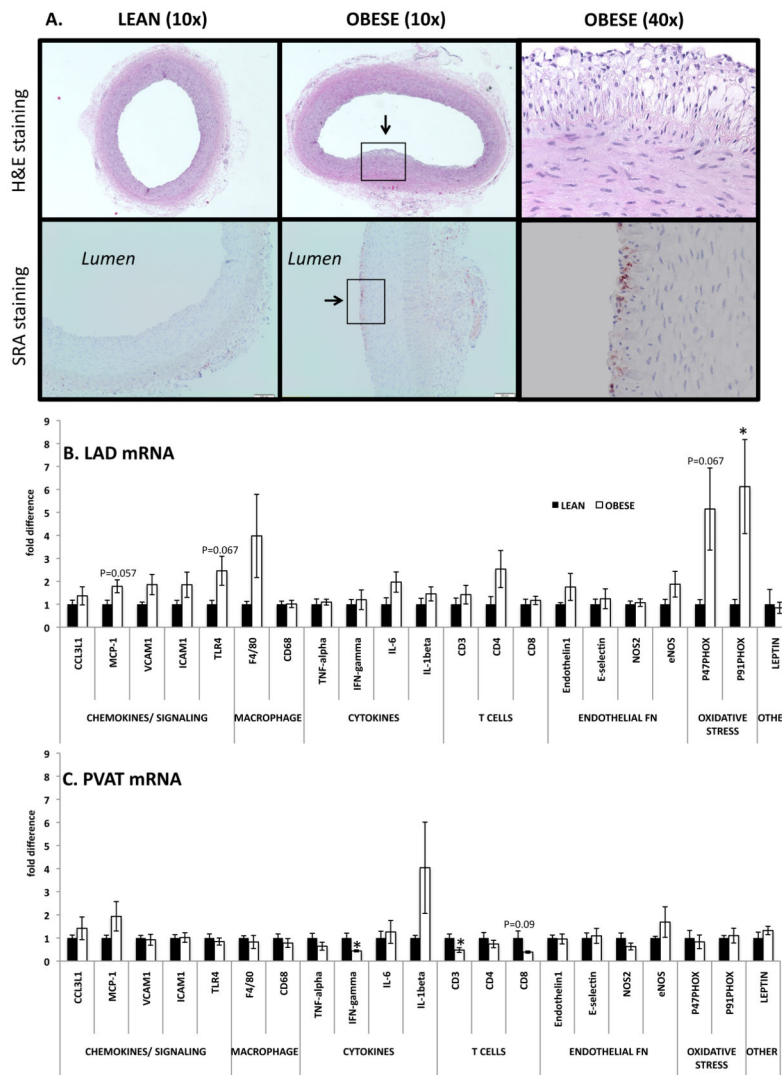


Figure 3. OBESSE pigs develop signs of coronary artery inflammation and atherosclerosis. A: Representative images of left anterior descending (LAD) coronary artery cross sections indicating lesion formation (indicated by arrow, top panels) and M ϕ staining (SRA staining, bottom panels; positive staining indicated by arrow) in OBESSE compared to LEAN; the last image in each row is the magnified region (40x) surrounded by the box in the second image which represents one OBESSE animal. B: Gene expression analysis of LAD. C: Gene expression analysis of AT surrounding the LAD (i.e., perivascular AT, PVAT). Data expressed as mean \pm SEM; * P<0.05.

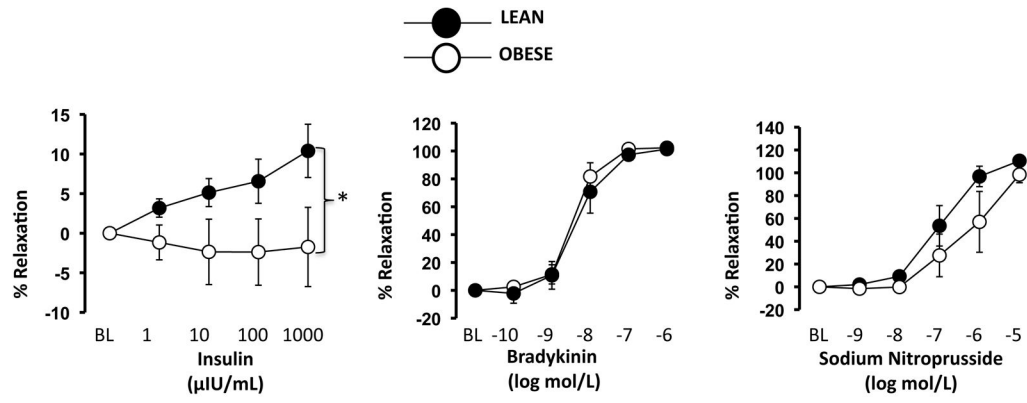


Figure 4. OBESE pigs exhibit impaired insulin-stimulated relaxation in left anterior descending (LAD) coronary artery
 Data expressed as mean ± SEM; * P<0.05.

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Table 1

Body composition and metabolic characteristics of LEAN and OBESE pigs

	LEAN (n=4)	OBESE (n=4)	P Value
Body weight, kg	37.3±1.51	100.4±2.00	0.0001
Length, inches	44.0±1.0	56.1±1.1	0.001
Percent body fat, %	29.2±3.13	41.9±1.15	0.009
Percent lean mass, %	72.0±3.6	56.6±0.9	0.006
Blood pressure (Systolic/Diastolic mmHg)	110±3/72±4	130±4/99±8	<0.05
Bone mass, kg	0.80±0.06	1.37±0.065	0.001
Heart mass, kg	0.13±0.013	0.20±0.008	0.001
Total cholesterol, mg/dl	80.0±5.64	189.5±35.2	0.022
LDL-c, mg/dl	31.5±1.5	104.0±21.3	0.014
HDL-c, mg/dl	40.5±3.80	56.25±3.80	0.026
LDL-c:HDL-c	0.79±0.06	1.80±0.3	0.007
Total cholesterol:HDL-c	1.99±0.07	3.29±0.4	0.015
NEFA, mmol/l	0.223±0.085	2.572±0.353	0.001
Triglycerides, mg/dl	27.5±7.5	77.5±14.0	0.02
Glucose, mg/dl	121±10.0	308±47.5	0.008
Insulin, µg/l	0.104±0.017	0.287±0.009	0.017
HOMA-IR	0.91±0.18	6.26±0.96	0.001
Adipo-IR	0.023±0.009	0.736±0.096	<0.001

Values are means ± SEM. LDL, low-density lipoprotein; HDL, high-density lipoprotein, NEFA, nonesterified fatty acids; HOMA-IR, homeostatic model assessment; Adipo-IR, adipocyte IR.