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CURRENT DEVELOPMENTS IN NUTRITION

Genomics, Proteomics, and Metabolomics

A Western-Type Dietary Pattern Induces an Atherogenic Gene Expression Profile in the Coronary Arteries of the Ossabaw Pig

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ABSTRACT

Background: Current cardiovascular risk reduction guidance focuses on shifts in dietary patterns, rather than single foods or nutrients. Experimental studies are needed to identify the mechanisms by which food-based diets affect the development and progression of atherosclerosis.

Objectives: The aim of this study was to investigate the effect of 2 food-based dietary patterns and statin therapy on the transcriptome of the left anterior descending coronary artery of the Ossabaw pig.

Methods: Pigs were randomly assigned to 1 of 4 groups and fed isocaloric diets for 6 mo; Heart Healthy–style diet (HHD) (high in unsaturated fat, unrefined grain, fruits/vegetables) or Western-style diet (WD) (high in saturated fat, cholesterol, refined grain), with or without atorvastatin. A 2-factor edge R analysis was used to determine differential gene expression in the left anterior descending coronary artery.

Results: Relative to the HHD, the WD resulted in the differential expression of 143 genes, of which 139 genes were upregulated and 4 genes were downregulated (all log fold change $\geq$0.6, false discovery rate $<$0.10). The WD, compared with the HHD, resulted in the statistically significant upregulation of 8 atherosclerosis-associated pathways implicated in immune and inflammatory processes. There were no genes with significant differential expression attributable to statin therapy.

Conclusions: These data suggest that a WD induces alterations in the transcriptome of the coronary artery consistent with an inflammatory atherogenic phenotype in the Ossabaw pig with no significant modification by concurrent statin therapy. *Curr Dev Nutr* 2019;3:nzz023.

Introduction

Poor diet quality is a key contributor in the development of coronary artery disease (CAD) (1). Observational and clinical research investigating diet-CAD relations have largely shifted focus from individual dietary components to food-based dietary patterns (2). This shift aligns with current guidance on cardiovascular risk that largely focuses recommendations on dietary patterns (3, 4). Food-based dietary patterns encompass the potential synergistic and cumulative effects of foods and are more reflective of habitual eating patterns (5). It is widely recognized that interplay between dietary patterns and progression or mitigation of CAD is complex and involves the alteration of many biological systems (6). Elucidating the molecular mechanisms by which food-based dietary patterns prevent, treat, or reverse the underlying pathology of CAD will help to better inform future guidance of population-based dietary recommendations to reduce risk.

The adoption of a healthy dietary pattern for CAD risk reduction is recommended prior to the initiation of statin therapy and should be maintained thereafter (7). Statins reduce plasma LDL

Keywords: Ossabaw pig, coronary artery disease, atherosclerosis, transcriptomics, dietary patterns, atorvastatin

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Abbreviations used: CAD, coronary artery disease; FDR, false discovery rate; HHD, Heart Healthy Diet; HHD + S, Heart Healthy Diet + atorvastatin; hsCRP, high-sensitivity C-reactive protein; LAD, left anterior descending; log FC, $\log_2$ fold change; WD, Western diet.

cholesterol concentrations primarily by inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase activity, which leads to lower cholesterol synthesis and increased hepatic expression of the LDL receptor, and are prescribed for both primary and secondary CAD risk reduction (8). In addition to lowering LDL cholesterol, statins also have anti-inflammatory properties (9). Few studies have examined the potential interactions between diet and statin therapies, but the limited data suggest synergistic effects (10, 11). The molecular mechanisms for this interaction are unexplored.

A major obstacle in the assessment of food-based dietary patterns on the development of CAD is the lack of appropriate animal models. Rodents are a poor translational model because they have lipoprotein profiles that are dissimilar to humans and, unless genetically modified or fed extreme diets, rarely develop atherosclerosis (12–14). Pigs offer a more representative animal model (12–15). Their cardiovascular anatomy and disease pathogenesis are like that of humans, and they are omnivorous, making them amenable to the study of food-based dietary patterns (12, 14). We have previously demonstrated that the Ossabaw miniature pig is a viable model for the study of food-based dietary patterns and CAD (16). Six months after feeding a Heart Healthy–style diet (HHD) and Western-style diet (WD), histologic evaluation in the right, left circumflex, and left anterior descending (LAD) coronary arteries indicated the presence of early atherosclerotic lesions. The incidence and severity of early atherosclerotic lesions was greater in pigs fed the WD (16). The objective of the present study was to investigate the effect of dietary patterns and statin therapy on the transcriptomic response of the LAD coronary artery. These data were then related to cardiometabolic risk indicators and atherosclerotic lesion severity.

Methods

Study design, animals, and diets

This work was conducted as an ancillary study and used a subset of 16 pigs described in a previously published investigation examining the effect of dietary patterns and statin therapy on atherosclerotic lesion development in the Ossabaw miniature pig (16). Pigs were obtained from the Ossabaw Research Unit (Indiana University School of Medicine, Indianapolis, IN). Research protocols and procedures were approved by the Beltsville Institutional Animal Care and Use Committee. Additional approval for biological sample/tissue storage and analysis was obtained from the Tufts Medical Center/Tufts University Institutional Animal Care and Use Committee. Initially 32 Ossabaw miniature pigs, aged 5–8 wk, were randomly assigned according to a 2 × 2 factorial design into 4 groups: HHD; HHD + atorvastatin (HHD + S); WD; and WD + atorvastatin (WD + S). The subset studied included 16 pigs (7 boars and 9 gilts): WD, $n = 4$; WD + S, $n = 4$; HHD, $n = 3$; and HHD + S, $n = 5$. The HHD and WD were designed to reflect human dietary patterns, and the animals were fed these diets in isocaloric amounts for 6 mo after 2 mo of acclimatization. Pigs from all groups were initially fed ∼2200 kcal/d. To support normal growth this amount was gradually increased to 5600 kcal/d over the course of the study (16). Details of the diet composition and food sources were reported previously (16). The diets provided 38% of energy (E) from fat, 47% E from carbohydrate, and 15% E from protein, but differed in the type of dietary fat and carbohydrate, and the amount of fiber and cholesterol (16). The HHD contained 29% E from unsaturated fats (canola, soybean, and corn oils); 9% E from saturated fat (anhydrous milk fat); 47% E from whole-wheat flour, oats, and a freeze-dried fruit/vegetable mix providing 13 g of fiber per 100 g of diet; and 0.1% wet weight of cholesterol. The WD contained 22% E from saturated fat (anhydrous milk fat); 16% E from unsaturated fat (canola, soybean, and corn oils); 47% E from sugar and white flour; 7 g of fiber per 100 g of diet; and 1.5% wet weight of cholesterol. Both diets contained 2.5% wet weight of a vitamin and mineral mix (17). Pigs in the HHD groups were supplemented with fish oil capsules [Epanova 1 g (550 mg eicosapentaenoic acid + 200 mg docosahexaenoic acid); AstraZeneca) 3 times/wk. Statin-treated pigs were given atorvastatin (Lipitor; Pfizer) at a dose of 20 mg/d during months 1–3 and 40 mg/d during months 4–6.

Sample collection

One HHD-fed pig died during the acclimatization period, and another pig (in the WD group) died during the baseline blood collection. Thirty pigs were killed at the conclusion of the study by intravenous injection with Euthasol (50 mg sodium pentobarbital/kg body weight; Virbac Animal Health, Inc.) after which blood and coronary artery tissue samples were collected. The killing, collection of blood, and fixation of samples for histology has been previously described (16). A 5-mm section of the LAD coronary artery was collected ∼5 mm from the aortic root and flash frozen in liquid nitrogen. Samples were stored at −80°C until processing.

Sample processing

Blood samples.

Concentrations of serum total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol and plasma high-sensitivity C-reactive protein (hsCRP) were measured as previously described (16). Briefly, concentrations of serum HDL cholesterol were measured on an AU400 clinical chemistry analyzer (Beckman Coulter, Inc.) and LDL cholesterol concentrations were estimated with the use of the Friedewald equation (18). Concentrations of plasma hsCRP were measured by a 2-site ELISA procedure (Pig High-Sensitive CRP ELISA cat. no. KT-184; Kamiya Biomedical Company).

Histology.

The histologic presence of atherosclerosis in the proximal LAD coronary artery was determined by a blinded board-certified veterinary cardiovascular pathologist and classified according to the Stary system (19).

Tissue RNA isolation.

Coronary artery tissue samples were homogenized, and the RNA was isolated with the use of an RNeasy Universal Mini kit (Qiagen) per the manufacturer's instructions. Isolated RNA was treated with Turbo DNase (Ambion) to minimize genomic DNA contamination. RNA quality was assessed with the use of an Experion RNA analysis electrophoresis kit (Bio-Rad). Samples with an RNA Quality Indicator >7 were sequenced.

TruSeq library preparation, sequencing, and processing of reads. Preparation for RNA sequencing was completed with the use of an Illumina TruSeq RNA Sample Preparation kit version 2 (Illumina) and AMPure XP beads (Beckman Coulter) as previously described (20). Experion DNA 1 K chips (Bio-Rad) were used to determine DNA fragment size. Library quantification was completed with the KAPA Library Quantification kit (KAPA Biosystems). Samples were sequenced with a NextSeq 500 sequencer (Illumina) with 100 basepair single-end reads. Raw data in FASTQ format was processed for quality with the use of the CLC Bio Genomic Workbench (Qiagen). The transcriptome was assembled with the use of the annotated *Sus scrofa* 10.2 as a reference genome (21) and was additionally checked against the porcine translational research database (22).

Transcriptome and gene enrichment analyses

Differential expression analysis was conducted with the use of the standard Bioconductor (23) package "edge R" which is based on a negative binomial distribution (24). Data were entered as raw gene counts, filtered based on a minimum of $\geq$1 count/million across 3 samples in all groups, and normalized with the use of the trimmed mean of M values method. One pig in the WD diet group had abnormally low counts across all genes, which may bias differential expression results, and was excluded from the analysis. A 2-factor edge R model was designed to determine differentially expressed genes attributable to the main effects of diet (WD compared with HHD) and statin (atorvastatin compared with no atorvastatin), and a diet $\times$ statin interaction. Differentially expressed genes were identified through the use of the Cox-Reid profile-adjusted likelihood method and likelihood ratio test. A Benjamini-Hochberg false discovery rate (FDR) was used to adjust *P* values for multiple comparisons (25). As this was an ancillary study conducted in a subset of 15 pigs, differentially expressed genes were defined as genes with an FDR-adjusted *P* value $<$0.10 and absolute $\log_2$ fold change (log FC) $\geq$0.60. A significant diet $\times$ statin interaction was indicated by an FDR-adjusted *P* value $<$0.1. Differentially expressed genes were subsequently analyzed with Ingenuity Pathway Analysis version 9.0 (IPA) to determine relevant biological pathways and functional annotation of differentially expressed genes. For pathway and functional annotation analyses a *z* score was calculated indicating up- or downregulation, and match to the Ingenuity Pathway Analysis Knowledge Base (observed compared with predicted). Pathways with an absolute *z* score $\geq$2 and an FDR-adjusted *P* value $\leq$0.05 were considered statistically significant. All raw RNA sequencing data was deposited in the Gene Expression Omnibus (GEO) repository (accession number GSE129300).

Further statistical analysis

Pigs from all groups were pooled ($n = 15$) and Spearman's correlation coefficients were calculated to determine associations between the expression of differentially expressed genes with concentrations of cardiometabolic risk indicators (LDL cholesterol, HDL cholesterol, and hsCRP) and atherosclerotic lesion severity. A random forest algorithm, produced by the R package MLSeq, was used to identify genes differentially expressed by the WD, with the top variable importance score based on ability to classify the pooled group of pigs by the presence of atherosclerosis in the proximal LAD coronary artery (26). Raw gene count data underwent a deseq normalization and voom transformation, and a random forest model was run with a *k*-fold cross-validation (CV = 10) and repeated 1000 times. The presence of atherosclerosis was a binary variable, with a Stary score of 0 indicating no presence of atherosclerosis, and 1 representing the presence of atherosclerosis (Stary score $\geq$1). The top 4 genes by variable importance score were used for correlation analysis. Due to the exploratory nature of the analysis correlation coefficients with a *P* value $\leq$0.05 were considered significant.

Results

Differential expression analysis

In total, the 2-factor analysis in edge R identified 143 genes with significant differential expression attributable to WD relative to HHD in the proximal LAD coronary artery. LogFC and biological processes for the top 15 annotated differentially expressed genes are presented in **Table 1** and a complete list of significant genes, expression levels, and their respective biological processes is provided in **Table S1**. The WD, compared with the HHD, resulted in the upregulation of 139 genes and the downregulation of 4. We identified 6 differentially expressed genes that were related to endogenous cholesterol homeostasis [oxidized LDL receptor (*LOX-1*, log FC = 5.0, FDR = 0.035) macrophage scavenger receptor (*MSR*, log FC = 4.7, FDR = 0.025), cytochrome P450 family 7 subfamily A member 1 (*CYP7A1*, log FC = 8.7, FDR = 0.004), ATP binding cassette subfamily A member 1 (*ABCA1*, log FC = 5.5, FDR = 0.002), apolipoprotein E (*APOE*, log FC = 4.6, FDR = 0.082), and phospholipid transfer protein (*PLTP*, log FC = 4.1, FDR = 0.069)]. No genes were differentially expressed by statin therapy or by a diet $\times$ statin interaction.

Gene enrichment analysis

Enrichment analysis was completed to determine the biological relevance of the 143 genes differentially expressed by the WD. Pathway analysis identified 8 statistically significant biological pathways that were upregulated by the WD, relative to the HHD (**Table 2**). Genes implicated in the identified pathways were involved in immune signaling [toll-like receptor 4 (*TLR4*), toll-like receptor (*TLR8*), triggering receptor expressed on myeloid cells 2 (*TREM*)], chemotaxis [integrin subunit β 2 (*ITGB2*) and selectin P ligand (*SELPLG*)], T cell activation [CD83 molecule (*CD83*) and CD86 molecule (*CD86*)] and extracellular matrix degradation [matrix metallopeptidase 9 (*MMP9*)]. Functional annotation analysis identified 161 statistically significant disease functions upregulated by the WD (**Supplementary Table 2**). The top disease functions identified were th following: *1*) immune response of cells (**Figure 1**), *2*) engulfment of cells, and *3*) phagocytosis. Differential expression and pathway analyses results were similar when the porcine translational research database was used (**Supplementary Tables 3** and **4**).

Associations of gene expression with cardiometabolic risk indicators and atherosclerosis

We sought to determine if the expression of 4 selected differentially expressed genes in the proximal LAD coronary artery was related to measures of cardiometabolic risk indicators or atherosclerotic lesion

TABLE 1 Top 15 Annotated Genes Differentially Expressed by the WD in the LAD Coronary Artery[1]

Gene symbol	Gene name	Log FC (WD vs HHD)	FDR	Biological processes
CYP7A1	Cytochrome P450 family 7 subfamily A member 1	8.7	0.004	Bile acid biosynthetic process; cellular response to cholesterol; cholesterol catabolic process
ATP6V0D2	ATPase H + transporting V0 subunit d2	8.3	0.002	ATP hydrolysis coupled proton transport; insulin receptor signaling pathway
TCN1	Transcobalamin 1	8.2	0.007	Cobalamin metabolic process; cobalamin transport; cobalt ion transport
TREH	Trehalase	8.0	0.006	α,α-Trehalase activity; catalytic activity; hydrolase activity
FAM59B	GRB2 associated regulator of MAPK1 subtype 2	7.9	0.005	—
C2ORF55	Chromosome 20 open reading frame 55	7.8	0.007	—
SLC45A3	Solute carrier family 45 member 3	7.7	0.007	Hexose transport; positive regulation of fatty acid biosynthetic process; positive regulation of glucose metabolic process
CD101	CD101 molecule	7.5	0.050	Cell surface receptor signaling pathway; positive regulation of myeloid leukocyte differentiation
MMP9	Matrix metallopeptidase 9	7.5	0.009	Cellular response to cadmium ion; cellular response to cell-matrix adhesion; cellular response to cytokine stimulus
FCRL1	Fc receptor–like 1	7.1	0.022	B cell activation; signal transduction
LYZ	Lysozyme	6.9	0.017	antimicrobial humoral response; cellular protein metabolic process; cytolysis; defense response to bacterium
TLR8	Toll-like receptor 8	6.8	0.078	cellular response to mechanical stimulus; defense response to virus; I-κB kinase/NF-κB cascade
P2RY13	Purinergic receptor P2Y13	6.6	0.060	Cellular response to organic cyclic compound; G-protein coupled purinergic nucleotide receptor signaling pathway; signal transduction
CLEC5A	C-type lectin domain containing 5A	6.5	0.070	Cellular defense response; immune system process; innate immune response; myeloid cell differentiation
TREM2	Triggering receptor expressed on myeloid cells 2	6.5	0.002	Dendritic cell differentiation; detection of lipopolysaccharide; innate immune response

[1] Biological processes are from the Ingenuity Pathway Analysis knowledge base. Note: differential expression attributable to the main effect of diet (WD, $n = 7$ compared with HHD, $n = 8$). LAD, left anterior descending; FC, fold change; HHD, Heart Healthy Diet; WD, Western diet; FDR, false discovery rate–adjusted *P* value.

severity as evaluated by Stary score (see Further Statistical Analysis section above). Least-squares means of LDL cholesterol, HDL cholesterol, and hsCRP concentrations, and mean atheromatous changes of Stary score in the proximal LAD coronary artery by pig group have previously been reported on the full sample of pigs (16). Here the analysis was limited to the subset of 15 pigs and was done independent of pig group. LDL cholesterol concentrations ranged from 32 to 657 mg/dL (median: 102 mg/dL), HDL cholesterol from 34 to 130 mg/dL (median: 70 mg/dL), and hsCRP from 43 to 114 mg/L (median: 74 mg/dL). Stary score in the proximal LAD coronary artery ranged from 0 to 2. Differentially expressed genes with the top variable importance score were *LOX-1*, phosphoinositide-3-kinase adaptor protein 1 (*PIK3AP1*), dedicator of cytokinesis 8 (*DOCK8*), and radical S-adenosyl methionine domain containing 2 (*RSAD2*). Expression of *LOX-1* was positively associated with hsCRP concentrations ($r = 0.54$, $P = 0.005$) and atherosclerotic lesion severity ($r = 0.54$, $P = 0.004$) (**Table 3**). *PIK3AP1* was positively associated with LDL cholesterol ($r = 0.72$, $P = 0.022$) and HDL cholesterol ($r = 0.60$, $P = 0.039$) concentrations. Expression of *DOCK8* was positively associated with atherosclerotic lesion severity ($r = 0.59$, $P = 0.021$).

Discussion

Despite current guidance for cardiovascular risk reduction focusing on shifts in dietary patterns rather than single foods or nutrients, there are few experimental data on the interplay between food-based dietary patterns and the development of coronary atherosclerosis or the molecular mechanisms mediating lesion progression. The present study was designed to address this gap by investigating the transcriptomic response of the LAD coronary artery of Ossabaw miniature pigs fed food-based HHD and WD, with or without statin therapy.

TABLE 2 Biological Pathways of Genes Differentially Expressed by the WD in the LAD Coronary Artery[1]

Pathways	Activation	Molecules	Signaling pathway categories	FDR
Dendritic cell mutation	Up	CD83, CD86, FCGR1A, LY75, PLCB2, TLR4, TNFRSF11B, TREM2	Cellular immune response, cytokine signalling, pathogen-influenced signaling	0.02
Production of nitric oxide and reactive oxygen species in macrophages	Up	APOE, CYBB, LYZ, NCF2, TLR4, TNFRSF11B	Cellular immune response	0.02
TREM1 signaling	Up	CD83, CD86, TLR4, TLR8	Cellular immune response; cytokine signaling	0.02
PI3K signaling in B lymphocytes	Up	C3, PIK3AP1, PLCB2, TLR4, VAV1	Cellular immune response	0.02
IL-8 signaling	Up	CYBB, ITGB2, MMP9, NCF2, PLCB2, PLD3	Cellular immune response, cytokine signaling	0.02
Leukocyte extravasation	Up	CYBB, ITGB2, MMP9, NCF2, SELPLG, VAV1	Cellular immune response	0.02
Neuroinflammation signaling	Up	CD86, CYBB, MMP9, NCF2, TLR4, TLR8, TREM2	Cellular immune response, disease-specific pathways, neurotransmitters and other nervous system signaling	0.02
NF-κB signaling	Up	PRKACB, TLR4, TLR8, TNFRSF11B, TNFSF13B	Cellular immune response, cytokine signaling, humoral immune response, organismal growth and development	0.04

[1]Note: pathway analysis was based on genes identified by the prior differential expression attributable to the main effect of diet (WD, $n = 7$ compared with HHD, $n = 8$). LAD, left anterior descending; WD, Western diet; FDR, false discovery rate–adjusted *P* value.

Differential gene expression and enrichment analyses indicated that the WD, relative to the HHD, induced gene expression consistent with an inflammatory atherogenic phenotype. Gene expression induced by the WD appeared to be indicative of the early stages of atherosclerotic lesion development in the proximal LAD coronary artery. Upregulated pathways and functional annotation of genes differentially expressed by the WD characterize many features in the development of atherosclerosis and lesion progression, including activation of endothelial cells, migration of immune cells to the endothelium, activation of macrophages, and the production of reactive oxygen species (27). The total enrichment analyses indicated an upregulation of innate, and potentially adaptive, immune responses associated with atherosclerosis in response to the WD relative to the HHD. These data are consistent with our previous histologic evaluation demonstrating the presence of early atherosclerotic lesions (intimal thickening and the presence of foam cells and macrophages) in the proximal and medial sections of the LAD coronary artery of WD-fed pigs (16).

In addition to these pathways, 6 genes involved in endogenous cholesterol homeostasis (*LOX-1*, *MSR1*, *ABCA1*, *PLTP*, *APOE*, and *CYP7A1*) were identified. *LOX-1* and *MSR1* are involved in the atherosclerotic process by the uptake of modified LDL cholesterol by macrophages which facilitates the internalization of LDL cholesterol into the arterial wall and the formation of foam cells (28). In contrast, both *ABCA1* and *PLTP* are involved in cholesterol efflux; transporting cholesterol to nascent HDL and facilitating the maturation of HDL (28, 29). Similarly, increased *APOE* expression has antiatherogenic effects such as increasing cholesterol efflux, reduced formation of oxidized LDL cholesterol, and anti-inflammatory effects (30–32). Lastly, there was a significant upregulation of *CYP7A1*, which is involved in cholesterol catabolism by conversion of cholesterol to bile acids and is primarily expressed in hepatic tissue in humans (33). In pigs, *CYP7A1* expression has been detected in multiple tissues, including liver, lung, ovary, and adipose tissue (34). To our knowledge, this is the first report of *CYP7A1* expression in porcine coronary tissue or immune cells. Previous examination of induced *CYP7A1* expression in macrophages reported an increased expression of *ABCA1* and rate of cholesterol efflux (33). Together, the increased expression of these genes suggests an increased uptake of oxidized LDL cholesterol and a compensatory increase in cholesterol efflux, which aligns with our previous report of a significant increase in HDL cholesterol of pigs fed the WD (16). However, it is important to note that the induction of genes related to cholesterol efflux by the WD was not sufficient to prevent lesion formation.

We observed no differential gene expression in the proximal LAD coronary artery by statin therapy and no significant diet × statin interactions. This aligns with our previous work in which we found no statistically significant effect of on the histologic assessment of coronary atherosclerosis, and may indicate that a higher dose of atorvastatin is needed to directly affect gene expression in the coronary artery (16).

Lastly, selected gene expression induced by the WD was positively associated with serum lipoprotein concentrations (*PIK3AP*), plasma hsCRP concentrations (*LOX-1*) and atherosclerotic lesion severity (*LOX-1* and *DOCK8*). These data suggest that perturbations in lipoprotein metabolism and systemic inflammation induced by the

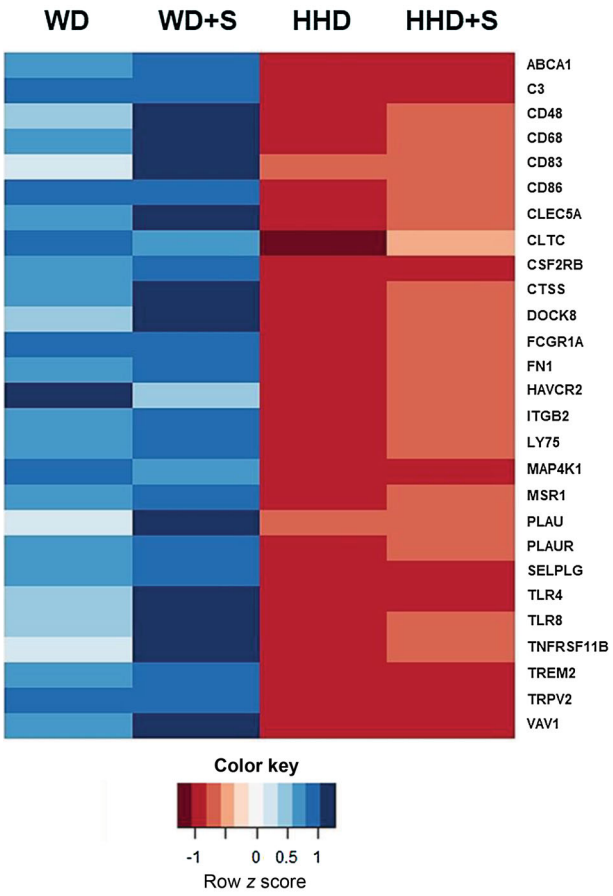

FIGURE 1 Average expression in reads per kilobase million (rpkm) *z* score of differentially expressed genes matched to the functional annotation of immune response of cells. HHD, Heart Healthy Diet; WD, Western diet; HHD + S, Heart Healthy diet with atorvastatin; WD + S, Western diet with atorvastatin; ABCA1, ATP binding cassette subfamily A member 1; C3, complement C3; CD48, CD48 molecule; CD68, CD68 molecule; CD83, CD83 molecule; CD86, CD86 molecule; CLEC5A, C-type lectin domain containing 5A; CLTC, clathrin heavy chain; CSF2RB, colony stimulating factor 2 receptor β common subunit; CTSS, cathepsin S; DOCK8, dedicator of cytokinesis 8; FCGR1A, Fc fragment of IgG receptor Ia; FN1, fibronectin 1; HAVCR2, hepatitis A virus cellular receptor 2; ITGB2, integrin subunit β 2; LY75, lymphocyte antigen 75; MAP4K1, mitogen-activated protein kinase kinase kinase kinase 1; MSR1, macrophage scavenger receptor 1; PLAU, plasminogen activator, urokinase; PLAUR, plasminogen activator, urokinase receptor; SELPLG, selectin P ligand; TLR4, toll-like receptor 4; TLR8, toll-like receptor 8; TNFSF13B, TNF superfamily member 13b; TREM2, triggering receptor expressed on myeloid cells; TRPV2, transient receptor potential cation channel, subfamily V, member 2; VAV1, vav guanine nucleotide exchange factor 1. Note: gene expression displayed by diet ± statin groups (WD, $n = 3$; WD + S, $n = 4$; HHD, $n = 3$; and HHD + S, $n = 5$.

WD likely facilitated or potentiated atherogenic gene expression in the LAD coronary artery. For example, *LOX-1* may have been induced by atherogenic stimuli including oxidized LDL cholesterol, and expression may be further potentiated by various inflammatory cytokines and CRP (35–37).

The strength of our study is the use of the Ossabaw miniature pig as a translational model for the investigation of human food-based dietary patterns. This experimental design allows for comprehensive assessment of how food-based dietary patterns may influence disease pathogenesis. Dietary guidance for CVD risk reduction now largely focuses on whole dietary patterns and macronutrient quality (3). However, experimental studies typically focus on macronutrient quantity. Our diets were formulated to reflect current guidance (HHD) and as much as possible a version of the current dietary pattern that does not meet the guidance (WD). This experimental design allows for a comprehensive assessment of how food-based dietary patterns of differing quality may influence pathogenesis. An additional strength is the replication of our transcriptomic and pathway analyses with the use of the porcine translational research database (22). This helps to ensure our results are robust to potential limitations in the annotation of the porcine genome. A limitation of the study is that gene expression was investigated in whole artery sections of the LAD coronary artery. Consequently, gene expression corresponds to the sum of endothelium, smooth muscle, adventitia, and immune cells, rather than a specific cell type. Large log FC values were observed in differentially expressed genes. This was likely due to low gene counts in some pigs (e.g., HHD-fed pigs) and suggests that the WD induced the expression of many differentially expressed genes. Direction of fold change and number of matched differentially expressed genes to biological pathways are better indicators of a diet effect than the magnitude of fold change. Transcriptomic analyses provide a descriptive assessment of gene expression, and although outside the scope of this study, a direction for targeted analyses on both the gene and protein expression. Although our study is exploratory in nature, and was completed on a subset of 15 pigs, the results are consistent with our previous histologic evaluation of atherosclerotic lesion severity. Lastly, there are limitations associated with the use of porcine models. Resources and logistics make it difficult to achieve a large sample size, or additionally to use mature pigs. It has been suggested that the Ossabaw pig may reflect a model of childhood rather than adult metabolic abnormalities (38). As the present study was conducted in immature Ossabaw pigs, the results may be limited to diet-induced changes in this development stage.

In conclusion, the present study used a translational pig model to investigate differences in the coronary artery transcriptome associated with food-based WD and HHD dietary patterns. Relative to the HHD, the WD induced an inflammatory atherogenic phenotype in the proximal LAD coronary artery, characterized by the development of atherosclerosis. Additional genes differentially expressed by feeding the WD suggested alterations in endogenous cholesterol homeostasis. Conversely, statin therapy had no statistically significant effect on the coronary artery transcriptome at the dosages used.

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TABLE 3 Associations of Gene Expression with Cardiometabolic Risk Factors and Atherosclerosis[1]

Gene	LDL cholesterol *r* (*P* value)	HDL cholesterol *r* (*P* value)	hsCRP *r* (*P* value)	Atherosclerosis *r* (*P* value)
LOX-1	0.52 (0.45)	0.41 (0.54)	0.54 (0.005**)	0.54 (0.004**)
PIK3AP1	0.72 (0.02*)	0.60 (0.04*)	0.28 (0.17)	0.44 (0.21)
DOCK8	0.30 (0.26)	0.21 (0.28)	0.14 (0.22)	0.59 (0.02*)
RSAD2	0.43 (0.08)	0.34 (0.10)	0.26 (0.33)	0.29 (0.18)

[1]Histologic evaluation of atherosclerosis by Stary score. Note: analysis conducted independent of diet ± statin ($n = 15$). *LOX-1*, oxidized LDL receptor; *PIK3AP1*, phosphoinositide-3-kinase adaptor protein 1; *DOCK8*, dedicator of cytokinesis 8; *RSAD2*, radical S-adenosyl methionine domain containing 2.
*$P < 0.05$; **$P < 0.01$.

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