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Western and heart healthy dietary patterns differentially affect the expression of genes associated with lipid metabolism, interferon signaling and inflammation in the jejunum of Ossabaw pigs

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Abstract

Diet quality and statin therapy are established modulators of coronary artery disease (CAD) progression, but their effect on the gastrointestinal tract and subsequent sequelae that could affect CAD progression are relatively unexplored. To address this gap, Ossabaw pigs ($N = 32$) were randomly assigned to receive isocaloric amounts of a Western-type diet (WD; high in

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Declaration of competing interest

We declare no competing interest.

Data statement

All raw RNA sequencing data from this manuscript will be available in the Gene Expression Omnibus (GEO) repository for public access (GSE160426).

Supplementary materials

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saturated fat, refined carbohydrate, and cholesterol, and low in fiber) or a heart healthy-type diet (HHD; high in unsaturated fat, whole grains, fruits and vegetables, supplemented with fish oil, and low in cholesterol), with or without atorvastatin, for 6 months. At the end of the study, RNA sequencing with 100 base pair single end reads on NextSeq 500 platform was conducted in isolated pig jejunal mucosa. A two-factor edgeR analysis revealed that the dietary patterns resulted in three differentially expressed genes related to lipid metabolism (*SCD, FADS1*, and *SQLE*). The expression of these genes was associated with cardiometabolic risk factors and atherosclerotic lesion severity. Subsequent gene enrichment analysis indicated the WD, compared to the HHD, resulted in higher interferon signaling and inflammation, with some of these genes being significantly associated with serum TNF-α and/or hsCRP concentrations, but not atherosclerotic lesion severity. No significant effect of atorvastatin therapy on gene expression, nor its interaction with dietary patterns, was identified. In conclusion, Western and heart healthy-type dietary patterns differentially affect the expression of genes associated with lipid metabolism, interferon signaling, and inflammation in the jejunum of Ossabaw pigs.

Keywords

Dietary patterns; Jejunum; Atherosclerosis; Cardiometabolic risk factors; Lipid; Ossabaw pig

1. Introduction

Approximately one in three deaths in the United States is attributed to cardiovascular diseases (CVD) [1]. Coronary artery disease (CAD), a type of CVD, is characterized by the presence of cholesterol-containing plaques exacerbated by dyslipidemia and inflammation, in the coronary arteries [1]. The gastrointestinal tract (GIT) is one point of control over cholesterol homeostasis, by modulating cholesterol absorption, which has subsequent effects on endogenous synthesis rates [2]. These evidence suggest a potential link between GIT function and atherogenesis, an area for which little data are currently available.

Adopting a heart healthy dietary pattern is the primary evidence-based lifestyle recommendation to prevent, treat, or reverse CAD [3–5]. A heart healthy dietary pattern emphasizes intake of vegetables, fruits, nuts, whole grains, and fish, while limiting intake of processed meats, refined carbohydrates, and sugar. This dietary pattern has been positively associated with a reduction in various CVD risk factors including plasma lipid and lipoprotein profiles, blood pressure, body weight, and waist circumference [6]. Numerous studies have assessed the relation between individual nutrients or food items and atherosclerosis, but the synergic effects contributed by the combined components of a heart health dietary pattern has for the most part been underinvestigated [7,8].

Individuals at elevated risk for CAD and demonstrating inadequate response to dietary modification are frequently treated with statins [3]. In addition to their LDL cholesterol-lowering effect, statins have pleiotropic effects, including antiproliferative, anti-inflammatory, and nitric oxide promoting actions [9]. Some evidence suggests these effects may modulate the GIT inflammatory response to dietary perturbations [9–11].

The Ossabaw pig is an animal model that develops diet-induced dyslipidemia and CAD [12]. The similarities between pig and human GIT anatomical structure and barrier defense mechanisms make this animal a good experimental model to study the relation between dietary patterns, GIT health, and atherosclerotic lesion development [13]. Our objective was to use a transcriptomic approach to characterize the effect of two dietary patterns, a heart healthy-type diet (HHD) and a Western-type diet (WD), with and without atorvastatin therapy (S), on jejunal mucosa gene expression in the Ossabaw pig. We hypothesized that the WD would result in higher permeability and inflammatory status of the jejunum, positively associated with atherosclerotic lesion development, and that atorvastatin therapy would mitigate these adverse diet-related effects.

2. Materials and methods

2.1. Study design and animals

This work was ancillary to a previously reported investigation designed to assess the effect of two dietary patterns, WD and HHD, with and without S, on the development of CAD in the Ossabaw pigs (Supplementary Fig. S1) [12]. Thirty-two Ossabaw pigs (16 boars + 16 gilts) were purchased from the Ossabaw Research Unit (Indiana University School of Medicine, Indianapolis, IN, USA). At 5–8 weeks of age the pigs were transferred to the Beltsville Agricultural Research Center (BARC) and randomly allocated into four groups using a 2 × 2 factorial design: WD-S, WD+S, HHD-S, HHD+S. Each group consisted of four boars and four gilts. After 1 month of acclimatization to a “grower diet” and another month of a gradual shift to the experimental diets, the pigs were fed their respective diets in isocaloric amounts, with a gradual increase in total energy to meet growth requirements, for 6 months. Two pigs died, unrelated to the experimental intervention, during the acclimatization period, resulting in a sample size of 30 pigs. Study protocols were approved by both the BARC and Tufts Medical Center/Tufts University Institutional Animal Care and Use Committee.

2.2. Diets and atorvastatin therapy

Isocaloric diets were designed to represent typical Western and heart healthy dietary patterns consumed by humans. Detailed diet composition and ingredient sources have been reported previously [12]. Briefly, both diets contained 38% of energy (E) as fat, 47% E as carbohydrate, and 15% E as protein. The major differences between the WD and HHD were the types of carbohydrate and fat, and amount of fiber, cholesterol, fruits, vegetables, and fish oil. The WD was rich in saturated fat (butter fat), cholesterol, and refined carbohydrate (sugar, white flour), and low in fiber, whereas the HHD was rich in unsaturated fat (canola, soybean, and corn oils), unrefined carbohydrate (whole wheat flour, oats), fruits/vegetables (freeze dried mix, Futureceuticals, Momence, IL, USA) and fiber, and low in cholesterol. Pigs fed the HHD were administered fish oil capsules (Epanova 1,000 mg [550 mg EPA + 200 mg DHA as free fatty acids], AstraZeneca, Cambridge, MA, USA) three times per week. Pigs in the atorvastatin (Lipitor, Pfizer, New York, NY, USA) groups were given 20 mg/d during the first 3 months and 40 mg/d during the latter 3 months of the intervention period.

2.3. Sample collection

At the end of the 6-month intervention period, the pigs were euthanized with an intravenous injection of Euthasol (50 mg sodium pentobarbital/kg body weight; Virbac Animal Health, Inc., Fort Worth, TX, USA). Jejunum segments (2 cm in length) were isolated, rinsed with ice-cold PBS, flash-frozen in liquid nitrogen, and stored at −80°C until processing. Blood samples were collected at necropsy as previously described [12].

2.4. Isolation of jejunal mucosa and RNA extraction

Frozen jejunum segments were incubated in pre-chilled RNA*later*-ICE (Invitrogen, Carlsbad, CA) at −20°C for 24 h, opened longitudinally, and the mucosa layer was cleanly separated from the muscle layer using a scalpel and tweezers. Total RNA was extracted from the jejunal mucosa using TRI Reagent according to the manufacturer's instructions (Zymo Research, Irvine, CA, USA), and treated with RNAseOUT (Invitrogen, Carlsbad, CA, USA) to minimize degradation, followed by TURBO DNA-free kit (Invitrogen, Carlsbad, CA, USA) to remove residual genomic DNA. The RNA quality and concentration were determined using Experion RNA Std-Sens Analysis kit (Bio-Rad, Hercules, CA, USA). All samples had an RNA Quality Indicator greater than 8 and were used for sequencing.

2.5. RNA sequencing

Illumina TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, CA, USA) and AMPure XP beads (Beckman Coulter, Hercules, CA, USA) were used to construct the sample libraries following manufacturers' protocols. Constructed libraries were quantified by KAPA Library Quantification kit (KAPA Biosystems, Wilmington, MA, USA). Fragment size of libraries was determined by Experion DNA 1K Analysis kit (Bio-Rad, Hercules, CA, USA). RNA sequencing was performed on NextSeq 500 platform (Illumina, San Diego, CA, USA) using NextSeq 500/550 Output kit v2.5 (Illumina, San Diego, CA, USA) with 100 base pair single end reads. The data output in FASTQ format were processed for quality trimming by CLC Bio Genomic Workbench (Qiagen, Valencia, CA, USA). The transcriptome was assembled and reconstructed using the domestic pig (*sus scrofa 11.1*) genome as reference [14]. Secondary analysis using a manually curated genome reference, the porcine translational research database [15], was also conducted to further validate the results.

2.6. Characterizing jejunal mucosal cell types and sample homogeneity

To assess consistency of jejunal mucosa sampling, the RNA sequencing data (reads per kilobase million) was analyzed using principal component analysis and hierarchical clustering (Spearman ranking method; RStudio, Version 1.0.153, Boston, MA, USA), following xCell analysis [16], a tool that utilizes transcriptome signals to predict enrichment of different cell types. On the basis of these analyses, one sample in the WD+S group was identified as an outlier and showed a high enrichment of preadipocytes and T-helper 1 cells relative to the other samples in the group and among all four groups, suggesting an error during sample collection. Hence, this sample was not included in subsequent analysis, resulting in a final sample size of 29 pigs. One-way analysis of variance (Prism 8, GraphPad Software, La Jolla, CA, USA) was used to analyze the epithelial cell enrichment data among

tissue samples among the four groups. No significant difference was identified among the four groups (Supplementary Fig. S2).

2.7. Differential expression analysis of RNA-seq data and gene enrichment analysis

Bioconductor package "edgeR" [17] was used to conduct differential expression analysis of genes using a two-factor model design matrix that identified differential gene expression attributable to dietary patterns, atorvastatin therapy, and their interaction (RStudio, version 1.0.153, Boston, MA, USA). Genes with a false discovery rate (FDR) <0.2 and absolute log fold change (logFC) ≥0.6 (absolute fold change ≥1.5) were considered differentially expressed. An exploratory pathway analysis was conducted: genes with an absolute log fold change ≥0.6 were uploaded to Ingenuity Pathway Analysis (IPA; v 9.0, Mountain View, CA, USA) to identify relevant biological pathways and functional annotations (known as Diseases & Functions). A *Z* score was calculated indicating up- or down-regulation of pathways or functional annotations by matching the dataset to IPA Knowledge Base (observed *vs.*predicted). Those with an absolute *Z* score ≥2 and an FDR adjusted *P* value ≤.05 was considered statistically significant.

2.8. Gene expression related to GIT permeability

A panel of annotated genes primarily related to GIT permeability were selected, including genes encoded for claudin proteins, tight-junction-associated MAR-VEL proteins, scaffolding proteins, and mucus formation [18]. Average expression of genes in each group was determined; logFC and FDR were calculated using method presented in Section 2.7.

2.9. Estimated desaturase activity

Fatty acid profiles were measured in fasting serum collected from the pigs at the end of the 6-month intervention period, as previously described [12]. The activity of stearoyl-CoA desaturases (SCD, enzyme encoded by *SCD* gene) were estimated using product/precursor ratios of serum fatty acids expressed in mol%, SCD_{16} – palmitoleate/palmitate (16:1n-7/16:0) and SCD_{18} – oleate/stearate (18:1n-9/18:0) [19–21]. The estimated activity of delta-5 desaturase (D5D, enzyme encoded by *FADS1* gene) was calculated based on the serum arachidonic acid/dihomo-gamma-linoleic acid (20:4 n-6/20:3 n-6) ratio.

Because there was no significant effect of statin therapy on desaturase gene expressions, the HHD and HHD+S, and WD and WD+S groups were combined for subsequent analyses. An unpaired two-tailed Student *t*test (Prism 8, GraphPad Software, La Jolla, CA, USA) was used to compare the estimated desaturase activities between the WD ($n = 14$) and the HHD ($n = 15$) groups. Differences were considered significant when $P \leq .05$.

2.10. Additional statistical analyses

To evaluate the association of genes expressed in jejunal mucosa with atherosclerotic lesions severity and cardiometabolic risk factors, pigs from the four groups were pooled ($n = 29$). Included in this analysis were three differentially expressed genes (*SCD, FADS1*, and *SQLE*), eight genes involved in altered IPA biological pathways (interferon signaling, phospholipase), and 17 genes identified in "inflammation of organ" functional annotation by IPA with certain predictive directions ("increased" or "decreased," but not "affected").

Spearman's correlation coefficients were calculated (Prism 8, GraphPad Software, La Jolla, CA, USA) to determine associations between gene expression and previously measured data [12] on atherosclerotic lesions severity (Stary score in left anterior descending-left circumflex bifurcation arteries) and serum cardiometabolic risk factors (LDL cholesterol, HDL cholesterol, triglycerides, tumor necrosis factor-alpha [TNF-α], and high-sensitivity C-reactive protein [hsCRP] concentrations). Due to the exploratory nature of the analyses, an absolute correlation coefficient $r \geq 0.4$ with a P value $\leq .05$ was considered statistically significant. Bonferroni correction was further used to adjust for multiple comparisons (168 comparisons, significant P value $\leq .0003$).

Although underpowered to evaluate sex differences, given the well-documented sex difference in CVD prevalence in humans [22] and our prior observations in the parent study [12], we performed a descriptive secondary analysis to determine whether there was a differential response of boars and gilts to the interventions using methods described in Section 2.7. Comparison analysis in IPA (v 9.0, Mountain View, CA, USA) was conducted to compare pathways and functional annotations altered by dietary patterns and atorvastatin therapy on the basis of sex.

3. Results

3.1. Differentially expressed genes

3.1.1. Dietary patterns—Three differentially expressed genes in the jejunal mucosa attributable to a dietary pattern effect were identified (Table 1). Pigs fed the WD, compared to HHD, had higher expression of stearoyl-CoA desaturase (*SCD*, logFC = 1.70, FDR = 0.14), and lower expression of fatty acid desaturase-1 (*FADS1*, logFC = −0.66, FDR = 0.14) and squalene epoxidase (*SQLE*, logFC = −1.21, FDR = 0.14).

3.1.2. Atorvastatin therapy—Folate hydrolase 1B gene expression was lower in the atorvastatin-treated pigs than nontreated pigs (*FOLH1B*, logFC = −3.68, FDR = 0.17). This difference appeared to be driven by two pigs in the HHD-S group that had expression of this gene that was 20times higher than the average expression in the other pigs in the group. A reversed trend was observed by excluding these two samples. Hence, no further interpretation will be made on this gene.

3.1.3. Dietary patterns x atorvastatin interaction—No significant interaction was observed between dietary patterns and atorvastatin therapy. Consequently, for data presentation and interpretation, WD-S and WD+S groups were combined and presented as WD; HHD-S and HHD+S were combined and presented as HHD.

3.2. Gene enrichment analysis

One hundred and forty-three genes that differed by dietary patterns with absolute log fold change of ≥0.6 were included in IPA gene enrichment analysis. Two significant biological pathways were identified that differed between the two dietary patterns (Table 2). The pigs in the WD groups exhibited higher interferon signaling pathway than the pigs in the HHD groups, attributable to four genes (interferon alfa inducible protein 6 [*IFI6*], interferon

induced protein with tetratricopeptide repeats 1 [*IFIT1*], ISG15 ubiquitin like modifier [*ISG15*], and MX Dynamin like GTPase 1 [*MX1*]). The pigs fed WD exhibited lower phospholipase pathway than the pigs in the HHD groups, attributable to four genes (lipase C hepatic type [*LIPC*], lipase G endothelial type [*LIPG*], phospholipase A2 group IID [*PLA2G2D*], and phospholipase B1 [*PLB1*]). Diseases & Functions analysis identified 10 significant functional annotations up-regulated and 7 down-regulated in pigs fed the WD, when compared to the HHD (Table 3). The most significant functional annotation module was higher "inflammation of organ" ($Z = 3.15$, $P < .001$) in the pigs fed the WD, compared to the HHD, in which 15 out of 17 genes predicted higher inflammation.

Gene enrichment analysis did not identify significant pathway or interpretable functional annotations alterations by atorvastatin therapy. Results from the differential expression and gene enrichment analyses were similar between the porcine translational research database and domestic pig database (Supplementary Tables S1–3) [15].

3.3. Gene expression related to GIT permeability

The expression of genes related to GIT permeability was similar regardless of dietary pattern or atorvastatin therapy (all FDR = 1, Supplementary Table S4).

3.4. Estimated desaturase activity

The estimated activity of SCD_{16} was significantly higher in the WD-than HHD-fed pigs ($P < .0001$, Table 4). In contrast, the estimated SCD_{18} activity was similar between the HHD- and WD-fed pigs ($P = .23$). WD-fed pigs had significantly lower estimated D5D activity than HHD-fed pigs ($P < .0001$).

3.5. Association of gene expression with atherosclerotic lesion severity and cardiometabolomic risk factors

Among the differentially expressed genes (Table 5), the expression of *SCD* gene in the jejunal mucosa was positively associated with atherosclerotic lesion severity, serum LDL and HDL cholesterol concentrations. *FADS1* gene expression was negatively associated with atherosclerotic lesion severity and serum TNF-α concentrations. *SQLE* expression was negatively associated with atherosclerotic lesion severity, serum LDL and HDL cholesterol concentrations.

Three genes (*IFI6, IFIT1*, and *ISG15*) involved in "Interferon Signaling" pathway (Table 5) were positively associated with serum TNF-α concentrations. Two genes involved in "Phospholipase" pathway were negatively associated with serum triglyceride concentrations. None of the genes involved in these pathways were significantly associated with atherosclerotic lesion severity.

For the identified genes in "inflammation of organ" functional annotation (Table 5), six anti-inflammatory genes were negatively associated with serum TNF-α (*ACKR1, GPX2, OLFM4, PGLYRP2, SELP*, and *SFTPD*) and/or hsCRP (*GPX2*) concentrations, and one pro-inflammatory gene was negatively associated with serum TNF-α (*TNFRSF21*) concentrations. Additionally, the gene expression of *GPX2* was negatively associated with

serum HDL concentrations; *LDLR* was negatively associated with atherosclerotic lesion severity and serum LDL cholesterol concentrations; *PLA2G2D* was negatively associated with serum triglyceride, LDL and HDL cholesterol concentrations; *SPP1* was negatively associated with serum triglyceride concentrations. Except for *LDLR*, no other inflammation-related genes were significantly associated with atherosclerotic lesion severity.

It should be noted that after Bonferroni correction adjustment, only four correlations remain significant: *SCD* with serum LDL concentrations, *SQLE* with atherosclerotic lesion severity, *LIPG* with serum hsCRP concentrations, and *LDLR* with atherosclerotic lesion severity.

3.6. Sex-specific trends

The impact of dietary patterns on pathways and functional annotation was similar in boars and gilts (Supplementary Fig. S3.1). Although when analyzed as a group there was no significant effect of atorvastatin therapy on jejunal gene expression, when the sexes were analyzed separately the pathways and functional annotations induced by atorvastatin therapy responded in different directions between the boars and gilts (Supplementary Fig. S3.2), suggesting there may be sex specific effects.

4. Discussion

Despite current guidelines for CVD risk reduction that emphasize overall dietary pattern modification rather than single nutrients or foods [5], limited evidence is available on the interplay between dietary patterns and CAD development, and the underlying molecular mechanisms thereof, particularly in the relation to the gut-arterial axis. This study assessed the effects of two dietary patterns, designed to mimic a WD and a HHD, with and without atorvastatin therapy, on gene expression in the jejunal mucosa of Ossabaw pigs and relate these data to atherosclerotic lesion severity. We found that dietary patterns altered gene expression associated with lipid metabolism (*SCD, FADS1,* and *SQLE*), and the expression of these genes was associated with arterial atherosclerotic lesion severity; yet we are unable to rule out the possibility of collinearity that dietary patterns altered jejunal gene expression as well as inducing atherosclerotic lesion. Gene enrichment analysis suggests that dietary patterns altered phospholipase pathway, interferon signaling pathway, and inflammation. No significant effect of atorvastatin therapy was observed, but data suggest that its effect on jejunal mucosa may differ by sex.

Two genes involved in fatty acid desaturation were differentially expressed when the two dietary patterns were compared. Higher expression of the *SCD* and lower *FADS1* gene was observed in the jejunal mucosa of pigs fed the WD, high in saturated fat, compared to the HHD, high in unsaturated fat [23]. This higher *SCD* gene expression induced by the WD was associated with higher estimated SCD16 activity, whereas estimated SCD18 activity was similar between the two dietary patterns. Prior work has suggested that SCD_{16} is a preferred marker for SCD activity due to its lower susceptibility to the dietary fatty acid profile [24,25]. Comparable observations were made in the duodenum of humans fed similar diets [26]. In a separate investigation in humans, a high compared to low saturated fat diet resulted in higher estimated SCD and lower D5D activities [27]. The *SCD* gene encodes for the stearoyl-CoA desaturase enzyme, a protein anchored in the endoplasmic reticulum

and responsible for the desaturation of saturated fatty acids, primarily converting stearate (18:0) to oleate (18:1n-9) and palmitate (16:0) to palmitoleate (16:1n-7) [28,29]. Our data are consistent with previous studies indicating that SCD gene expression is responsive to diet modification [30,31]. In mouse liver, *SCD* gene expression was upregulated by diets high in carbohydrate, saturated fat or cholesterol, and was downregulated by a diet high in polyunsaturated fat [32–36]. High hepatic SCD activity has been observed in several disease states, including obesity, atherosclerosis, diabetes, and cancer in humans and animal models [37–39]. Despite abundant investigations of hepatic SCD expression, the regulation of this gene in the small intestine is not well-understood [40]. In our study, *FADS1* gene expression was higher in pigs fed the HHD compared to the WD in the jejunal mucosa, and these data were consistent with the estimated D5D enzyme activity in serum. The *FADS1* gene encodes for the delta-5 desaturase protein that converts dihomo-gamma-linoleic acid (20:3 n-6, DGLA) to arachidonic acid (20:4 n-6, ARA) and eicosatetraenoic acid (20:4 n-3, ETA) to eicosapentaenoic acid (20:5 n-3, EPA) [41]. Altered estimated enzyme activity or expression of *FADS1* gene has been implied in several disease states, including CAD and insulin resistance [42,43]. Most studies that have investigated fatty acid metabolism in the small intestine focused on absorption, esterification and chylomicrons formation, rather than desaturation activities [40]. Our data suggest that dietary patterns altered gene expression associated with fatty acids desaturation in the jejunal mucosa.

Interferon plays a key role in the regulation of the innate and adaptive immune response to invading pathogens [44,45]. Our data suggest that interferon signaling in the jejunal mucosa was up-regulated by the WD compared to the HHD. Saturated fatty acids, higher in the WD, can act as agonists for toll-like receptors (TLR), specifically TLR2 and TLR4, which in turn activate type I interferon signaling [46–48]. Palmitic acid has been reported to induce type I interferon expression in macrophages and hepatocytes [49]. Our group has previously demonstrated that the WD upregulated interferon signaling, relative to the HHD, in epicardial adipose tissue [50]. Lower expression of *SQLE*, the gene encoded for a rate-limiting enzyme in cholesterol biosynthesis [51], was observed in the jejunal mucosa of pigs fed the WD compared to the HHD. The GIT plays an important role in regulating cholesterol homeostasis by modulating cholesterol bioavailability, which in turns influences LDL receptor activity and endogenous cholesterol synthesis rates [2]. There is an inverse relation between rates of cholesterol absorption and synthesis. Since *SQLE* is regulated at the transcriptional level by cellular sterol concentrations [52], it is likely that the lower *SQLE* expression in response to the WD compared to the HHD is mediated by the higher cholesterol content in WD. *In vitro* studies suggested that interferon signaling resulted in reduction in cellular sterol biosynthesis, and this reduction may further increase interferon signaling [53,54]. In our study, the altered gene signatures by dietary patterns also support this interferon-sterol relationship in the porcine jejunal mucosa.

IPA Diseases & Functions analysis predicted that the WD compared to the HHD had a higher degree of inflammation in jejunal mucosa. This prediction was based on comparing the jejunal RNA sequencing data with IPA Knowledge Base. For example, IPA Knowledge Base identified 14 matching results that consistently found surfactant protein D (*SFTPD)* an anti-inflammatory gene. As the WD-fed pigs had lower expression of this gene compared to the HHD, IPA predicted higher inflammation in the WD group. We found that 6 out of

15 genes (40%) in this category were significantly associated with systemic inflammatory biomarkers, suggesting local inflammation induced by dietary patterns in the jejunal mucosa is consistent with its effect on systemic inflammation. It should be noted that although two genes predicted lower inflammation in the WD than the HHD-fed pigs, the predictions were based on very limited prior investigations, hence, with low confidence.

Although we found that the expression of a substantial number (10) of genes involved in "Interferon Signaling" pathway and "Inflammation of Organ" functional annotation was significantly associated with systemic inflammation markers, the vast majority (nine) of these genes were not significantly associated with the primary end point, atherosclerotic lesion severity. These data suggest that diet-induced interferon signaling and local inflammation in jejunal mucosa are unlikely to be the mediators for atherosclerotic lesion development. Alternatively, the genes altered by dietary patterns and that were significantly association with atherosclerotic lesion severity, *SCD, FADS1, SQLE,* and *LDLR*, are all involved with lipid metabolism. Similar associations were observed between these differentially expressed genes and serum LDL and HDL cholesterol concentrations. These associations suggested that jejunal genes involved in lipid metabolism induced by the WD, high in cholesterol relative to the HHD diet, may have promoted or altered cholesterol homeostasis to promote atherosclerotic lesion progression. For example, lower expression of *LDLR* induced by WD in the jejunal mucosa may have resulted in lower cholesterol excretion, lead to higher serum LDL cholesterol concentrations, and exacerbate atherosclerotic lesion formation [55]. The higher levels of dietary cholesterol entered the small intestine may have exacer-bated this response.

To the best of our knowledge, this is the first study using a translational animal model to assess the relationship among dietary patterns, atorvastatin therapy and intestinal health. Dietary pattern-based interventions allow for the study of diet from a holistic rather than individual nutrient perspective, and take the complexity of "*dark matter of nutrition*" into account [56]. One of the limitations of our study was that, although the mucosa layer was cleanly separated from muscle layer, RNA was isolated from mucosal tissue homogenates: these samples reflect multiple cell types and may have diluted the true signals. To take this into account, we relaxed the FDR criteria when conducting the gene enrichment analysis. The RNA sequencing methodology also did not allow for the characterization of gene expression in specific cell types. Due to the nature of the current study, we were unable to verify the causality between jejunum physiology and development of atherosclerotic lesion severity, not explain the apparent disassociation between obesity, metabolic syndrome, inflammation and atherosclerosis, often observed in humans. In addition, we were not able to directly assess gut permeability other than evaluating gene expression in this study. A modest beneficial effect of atorvastatin therapy was observed on atherosclerotic lesion formation, regardless of dietary pattern [12]. However, no significant effect was observed on the jejunum measures. We cannot rule out the possibility that this was related to the atorvastatin doses used. Of note, the doses ware chosen to mimic typically prescribed human dosage, consistent with the translational goal of the study, and concern about potential side effects previously reported in pigs at higher doses [12].

In conclusion, relative to pigs fed the HHD, pigs fed the WD had higher *SCD* gene expression and lower *FADS1* and *SQLE* gene expression in the jejunal mucosa. Although the expression of these genes was associated with the severity of atherosclerotic lesions and serum lipoprotein cholesterol concentrations, it is possible that these observations were due to collinearity. Jejunal gene expression of *SCD* and *FADS1* was consistent with estimated SCD and D5D enzyme activities in serum. Although the WD resulted in higher interferon signaling and inflammation relative to the HHD and is consistent with the effect on systemic inflammation, these alterations were not significantly associated with atherosclerotic lesion severity. Genes associated with jejunum permeability were unaffected by dietary patterns or atorvastatin therapy. Although atorvastatin therapy decreased serum LDL cholesterol concentrations as previously reported [12], it had no significant effect on gene expression in the jejunal mucosa.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Genes differentially expressed in the jejunal mucosa of Ossabaw pigs fed the WD relative to HHD.*

Gene symbol	Gene name	logFC (WD *vs.* HHD)	FDR	Biological processes
SCD	Stearoyl-CoA desaturase	1.70	0.14	Cholesterol esterification; defense response to Gram-positive bacterium; fatty acid biosynthetic process; fatty acid metabolic process; fatty-acyl-CoA biosynthetic process; lipid biosynthetic process; lipid metabolic process; regulation of cholesterol biosynthetic process; response to fatty acid; unsaturated fatty acid biosynthetic process;
FADS1	Fatty acids desaturase 1	–0.66	0.14	Alpha-linolenic acid metabolic process; arachidonic acid metabolic process; fatty acid biosynthetic process; fatty acid metabolic process; eicosanoid biosynthetic process; linoleic acid metabolic process; lipid metabolic process; response to insulin stimulus; response to sucrose stimulus; unsaturated fatty acid biosynthetic process
SQLE	Squalene epoxidase	–1.21	0.14	Cholesterol biosynthetic process; cholesterol metabolic process; regulation of cholesterol biosynthetic process; sterol biosynthetic process

FDR, false discovery rate-adjusted *P* value; logFC, log fold change; HHD, heart healthy-type diet; WD, Western-type diet.

*Differential expression attribute to the main effect of dietary patterns (WD, $n = 14$; HHD, $n = 15$); Biological processes are from IPA Knowledge Base.

Table 2

Biological pathways affected by dietary patterns in the jejunal mucosa of Ossabaw pigs fed the WD relative to HHD*

Pathways	Activation	Molecules	Signaling pathway categories	FDR
Interferon signaling	Up	IFI6, IFIT1, ISG15, MX1	Cellular immune response; cytokine signaling	0.0069
Phospholipase	Down	LIPC, LIPG, PLA2G2D, PLB1	Carbohydrate metabolism; cell death and survival; organismal survival	0.0215

FDR, false discovery rate-adjusted *P* value; HHD, heart healthy-type diet; WD, Western-type diet.

*Pathway analysis was based on 143 genes differentially expressed by the WD relative to the HHD, with absolute log fold change of ≥0.6 (WD, $n = 14$; HHD, $n = 15$).

Table 3

Disease & function analysis in the jejunal mucosa of Ossabaw pigs fed the WD relative to HHD*

Diseases or functions annotation	FDR-adjusted *P* value	IPA predicted activation state	Activation *Z* score	Molecules
Inflammation of organ	.00004	Increased	3.15	ACKR1, ADA, CDA, CKM, CSF3R, CXCL2, DMBT1, GCNT3, GPX2, HBB, HERC5, IFI44, KCNH6, LDLR, LECT2, MMP9, MX1, NOS2, OLFM4, PGLYRP2, PLA2G2D, SCD, SELP, SERPINB5, SFTPD, SPP1, TGM3, TNFRSF21, TNFSF15, VIP
Inflammation of body cavity	.00021	Increased	3.13	ACKR1, ADA, CKM, CSF3R, CXCL2, DMBT1, GCNT3, GPX2, HERC5, IFI44, KCNH6, LDLR, LECT2, MMP9, NOS2, OLFM4, PGLYRP2, SCD, SELP, SFTPD, SPP1, TNFSF15, VIP
Inflammation of absolute anatomical region	.00085	Increased	2.80	ACKR1, ADA, CKM, CSF3R, CXCL2, DMBT1, GCNT3, GPX2, HERC5, IFI44, KCNH6, LDLR, LECT2, MMP9, NOS2, OLFM4, PGLYRP2, SCD, SELP, SFTPD, SPP1, TNFRSF21, TNFSF15, VIP
Lipolysis	.00016	Decreased	−2.45	ADA, LDLR, LIPC, LIPG, NOS2, PLA2G2D, SNCG, SREBF1, VIP
Concentration of phospholipid	.00028	Increased	2.42	ADA, LDLR, LIPC, LIPG, SCD, SFTPD, SPP1, SREBF1
Hydrolysis of lipid	.00223	Decreased	−2.40	ADA, LDLR, LIPC, LIPG, PLA2G2D, VIP
Migration of myeloid cells	.00003	Decreased	−2.37	ACKR1, CCL19, CXCL2, DMBT1, LDLR, MMP9, NOS2, SELP, SPP1
Dehydration of mice	.00010	Increased	2.24	ALOXE3, ARNTL, NEUROG3, NOS2, PKP1
Cleavage of carbohydrate	.00395	Decreased	−2.24	LIPC, LIPG, MMP9, PLA2G2D, VIP
Quantity of LDL cholesterol in blood	.00003	Increased	2.22	ARNTL, LDLR, LIPC, LIPG, NOS2
Dermatitis	.00158	Increased	2.21	ADA, HBB, LDLR, MX1, NOS2, PGLYRP2, PLA2G2D, SELP, SERPINB5, SFTPD, TGM3
Blood pressure	.00134	Increased	2.21	ARNTL, CKM, DBP, LDLR, MMP9, NOS2, SPP1, TEF, VIP
Uptake of lipid	.00213	Decreased	−2.20	ACAT2, LDLR, LIPC, NEUROG3, SFTPD, SREBF1
Concentration of triacylglycerol	.00001	Increased	2.17	ACAT2, ARNTL, GCKR, LDLR, LIPC, LIPG, MLXIPL, NOS2, PFKFB1, PPP1R3C, SCD, SPP1, SREBF1
Quantity of eosinophils	.00217	Increased	2.10	ADA, ARNTL, MMP9, NOS2, SELP
Binding of myeloid cells	.00189	Decreased	−2.06	BPI, CSF3R, CXCL2, LIPG, NOS2, SELP, SFTPD
Binding of professional phagocytic cells	.00128	Decreased	−2.06	BPI, CSF3R, CXCL2, LIPG, NOS2, SELP, SFTPD

HHD, heart healthy-type diet; WD, Western-type diet.

*Functional annotation analysis was based on 143 genes differentially expressed by the WD relative to the HHD, with absolute log fold change of ≥0.6 (WD, $n = 14$; HHD, $n = 15$).

Table 4

Estimated desaturase activities in the serum of Ossabaw pigs fed the WD and HHD*

Estimated desaturase	Dietary patterns		
	WD	HHD	*P*
SCD_{16}	0.11 (0.10, 0.13)	0.036 (0.032, 0.041)	<.0001
SCD_{18}	1.53 (1.35, 1.70)	1.37 (1.15, 1.59)	.23
D5D	5.59 (4.60, 6.58)	22.12 (15.00, 29.24)	<.0001

D5D, estimated delta-5 desaturase activity based on serum arachidonic acid/dihomo-gamma-linoleic acid (20:4 n-6/20:3 n-6) mol%; HHD, heart healthy-type diet; SCD_{16}, estimated stearoyl CoA desaturase activity based on serum palmitoleate/palmitate (16:1n-7/16:0) mol%; SCD_{18}, estimated stearoyl CoA desaturase activity based on serum oleate/stearate (18:1n-9/18:0) mol%; WD, Western-type diet.

*Values are means (95% confidence intervals) compared with the use of two-tailed Student *t* test by the main effect of dietary patterns (WD, $n = 14$; HHD, $n = 15$).

Table 5

Association of gene expression with atherosclerotic lesion and cardiometabolomic risk indicators*

		Atherosclerotic lesion severity	LDL cholesterol	HDL cholesterol	Triglyceride	TNF-α	hsCRP
Gene symbol	**Gene name**	***r* (*P* value)**	***r* (*P* value)**	***r* (*P* value)**	***r* (*P* value)**	***r* (*P* value)**	***r* (*P* value)**
Differentially expressed genes†							
SCD	Stearoyl-CoA desaturase	0.43 (.02)//	O.68 (<.0003)¶	0.55 (.002)//	0.30 (.12)	–0.02 (.94)	0.28 (.14)
FADS1	Fatty acids desaturase 1	–0.58 (.001)//	–0.29 (.12)	–0.19 (.32)	0.36 (.06)	–0.44(.03)//	–0.30 (.11)
SQLE	Squalene epoxidase	–0.75 (<.0 003)¶	–0.55(.002)¶	–0.39(.04)//	0.20 (.32)	–0.30 (.14)	–0.30 (.11)
Genes in “Interferon Signaling” pathway‡							
IFI6	Interferon alfa inducible protein 6	0.33 (.08)	0.01 (.98)	–0.17 (.38)	–0.33 (.09)	0.47 (.02)//	0.24 (.21)
IFIT1	interferon induced protein with tetratricopeptide repeats 1	0.10 (.61)	–0.26 (.18)	–0.29 (.12)	–0.20 (.30)	0.42 (.04)//	0.28 (.14)
ISG15	1SG15 ubiquitin like modifier	0.15 (.44)	–0.12 (.54)	–0.20 (.30)	–0.25 (.20)	0.52 (.008)//	0.19 (.33)
MX1	MX Dynamin like GTPase 1	0.22 (.26)	0.01 (.95)	–0.19 (.33)	–0.21 (.27)	0.26 (.21)	0.17 (.37)
Genes in “Phospholipase” pathway‡							
LIPC	Lipase C hepatic type	0.03 (.87)	–0.08 (.67)	–0.06 (.77)	–0.50 (.007)//	–0.04 (.83)	–0.11 (.57)
LIPG	Lipase G endothelial type	–0.35 (.07)	–0.20 (.30)	–0.35 (.06)	–0.07 (.73)	–0.32 (.12)	–0.66 (<.0003)
PLA2G2D	Phospholipase A2 group IID	–0.25 (.19)	–0.60 (.001)//	–0.50 (.006)//	–0.5 (.001)//	0.09 (.66)	–0.25 (.19)
PLB1	Phospholipase 81	–0.06 (.75)	–0.07 (.71)	–0.26 (.17)	0.14 (.47)	–0.27 (.20)	–0.26 (.17)
Genes in “Inflammation of Organ” functional annotation§							
ACKR1	Atypical chemokine receptor 1	–0.24 (.20)	0.00 (.98)	–0.15 (.43)	0.11 (.56)	–0.54 (.005)//	–0.30 (.11)
ADA	Adenosine deaminase	–0.35 (.06)	–0.15 (.44)	–0.15 (.45)	–0.01 (.96)	0.01 (.98)	0.26 (.18)
DMBT1	Deleted in malignant brain tumors 1	–0.03 (.89)	0.14 (.46)	–0.04 (.82)	0.02 (.90)	–0.30 (.15)	–0.10 (.61)
GCNT3	Glucosaminyl (*N*-acetyl) transferase 3	–0.16 (.39)	–0.07 (.70)	–0.08 (.69)	0.12 (.53)	0.36 (.07)	–0.14 (.47)
GPX2	Glutathione peroxidase 2	–0.16 (.40)	–0.17 (.38)	–0.40 (.03)//	–0.11 (.58)	–0.45 (.03)//	–0.47 (.01)//
LDLR	Low-density lipoprotein receptor	–0.64 (<.0003)	–0.60 (<.001)//	–0.39 (.04)	0.00 (.98)	–0.20 (.34)	–0.39 (.04)

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		Atherosclerotic lesion severity	LDL cholesterol	HDL cholesterol	Triglyceride	TNF-α	hsCRP
Gene symbol	**Gene name**	***r* (*P* value)**	***r* (*P* value)**	***r* (*P* value)**	***r* (*P* value)**	***r* (*P* value)**	***r* (*P* value)**
LECT2	Leukocyte cell derived chemotaxin 2	0.02 (.92)	–0.03 (.87)	0.03 (.87)	–0.03 (.90)	0.09 (.68)	0.02 (.93)
NOS2	Nitric oxide synthase 2	–0.18 (.35)	–0.06 (.76)	–0.30 (.12)	0.04 (.83)	–0.26 (.22)	–0.33 (.08)
OLFM4	Olfactomedin 4	–0.19 (.32)	–0.06 (.74)	–0.16 (.42)	–0.08 (.68)	–0.51 (.01)//	–0.35 (.07)
PGLYRP2	Peptidoglycan recognition protein 2	–0.32 (.09)	–0.11 (.56)	–0.19 (.32)	0.12 (.54)	–0.40 (.05)//	–0.29 (.13)
PLA2G2D	Phospholipase A2 group IID	–0.25 (.19)	–0.60 (.001)//	–0.50 (.006)//	–0.58 (.001)//	0.09 (.66)	–0.25 (.19)
SELP	Selectin P	–0.15 (.44)	0.17 (.38)	0.04 (.85)	0.27 (.17)	–0.62 (.001)//	–0.29 (.13)
SFTPD	Surfactant protein D	0.00 (1.00)	–0.07 (.70)	–0.18 (.35)	–0.23 (.23)	–0.44 (.03)//	0.02 (.93)
SPP1	Secreted phosphoprotein 1	0.27 (.15)	–0.11 (.55)	–0.16 (.42)	–0.60 (<.001)//	0.26 (.21)	0.32 (.09)
TNFRSF21	TNF receptor superfamily member 21	–0.03 (.87)	0.19 (.32)	–0.05 (.78)	0.23 (.24)	–0.49(.01)//	–0.14 (.48)
TNFSF15	TNF superfamily member 15	0.09 (.64)	–0.09 (.66)	0.09 (.64)	–0.06 (.76)	0.31 (.13)	0.31 (.10)
VIP	Vasoactive intestinal peptide	–0.24 (.22)	–0.03 (.88)	–0.11 (.56)	0.11 (.59)	–0.05 (.81)	–0.11 (.57)

hsCRP, high-sensitivity C-reactive protein; TNF-α, tumor necrosis factor-alpha.

*Analysis conducted independent of treatments (n = 29, except for triglyceride [n = 28] and TNF-α [n = 25]). Atherosclerotic lesion severity was assessed by Stary score in left anterior descending-left circumflex bifurcation arteries.

†Differentially expressed genes altered by dietary patterns.

‡Identified genes in biological pathways (by IPA) altered by dietary patterns.

§Identified genes in "inflammation of organ" module of Disease & Function analysis (by IPA) altered by dietary patterns. Genes with certain predictive directions ("increased" or "decreased," but not "affected") were included.

//Absolute correlation coefficient r ≥ 0.4, $P \leq .05$.

¶Absolute correlation coefficient r ≥ 0.4, $P \leq .0003$ (after Bonferroni correction).