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Dietary patterns influence epicardial adipose tissue fatty acid composition and inflammatory gene expression in the Ossabaw pig^{★,★★}

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Abstract

Epicardial adipose tissue (EAT) inflammation is implicated in the development and progression of coronary atherosclerosis. Dietary saturated and polyunsaturated fatty acids (SFAs and PUFA) can influence adipose tissue inflammation. We investigated the influence of dietary patterns, with emphasis on dietary fat type, and statin therapy, on EAT fatty acid (FA) composition and inflammatory gene expression. Thirty-two Ossabaw pigs were fed isocaloric amounts of a Heart Healthy (high in unsaturated fat) or Western (high in saturated fat) diets +/- atorvastatin for 6 months. EAT FA composition reflected dietary fat composition. There was no significant effect of atorvastatin on EAT FA composition. Total and long-chain SFAs were positively associated with inflammatory signaling (*TLR2*) and a gene involved in lipid mediator biosynthesis (*PTGS2*) ($P < .0003$). Medium-chain SFAs capric and lauric acids were negatively associated with *IL-6* (all $P < .0003$). N-6 and n-3 PUFAs were positively associated with anti-inflammatory signaling genes (*PPARG*, *FFAR4* and *ADIPOQ*) and long-chain n-3 PUFAs were positively associated with a gene involved in lipid mediator biosynthesis (*ALOX5*) (all $P < .0003$). These data indicate that dietary patterns, differing in fat type, influence EAT FA composition. Associations between EAT SFAs, PUFAs, and expression of genes related to inflammation provide a link between dietary quality and EAT inflammation.

Keywords

Epicardial adipose tissue; Fatty acids; Dietary patterns; Dietary fat; Ossabaw; Atorvastatin

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jnutbio.2019.04.013>.

1. Introduction

Chronic low-grade inflammation is thought to be a key mediator in the association between obesity and the development of cardiometabolic diseases [1]. Adipose tissue dysfunction, characterized by hypoxia, immune cell infiltration, increased free fatty acid release, and increased production of pro-inflammatory adipocytokines, is a key source of inflammation [1]. Epicardial adipose tissue (EAT) is an adipose tissue depot adjacent to the coronary arteries and in direct contact with the myocardium [2]. It is hypothesized that EAT with this inflammatory phenotype acts locally on the underlying coronary arteries, potentiating the development of coronary artery disease (CAD) [2]. Evidence supporting this hypothesis in humans includes positive associations of EAT volume with coronary artery calcification and plaque severity, as well as a higher expression of pro-inflammatory cytokines and macrophage infiltration in EAT from individuals with CAD, than those without CAD [3–5]. Thus, modulation of EAT inflammation is considered a potential therapeutic target for the management of CAD [6].

Fatty acids can alter adipose tissue inflammation via direct/indirect changes in gene expression and through their metabolism to lipid mediators [7,8]. Saturated fatty acids (SFAs) bind to surface toll-like receptors (*TLRs*) and trigger an innate inflammatory response and the production of pro-inflammatory adipocytokines [9]. SFAs have also been found to activate the NOD-like receptor pyrin domain containing 3 (*NLRP3*) inflammasome [10,11]. In contrast, long-chain n-3 polyunsaturated fatty acids (PUFAs) reduce adipose tissue inflammation. N-3 PUFAs, modulate inflammatory gene expression by signal transduction leading to the inhibition of pro-inflammatory transcription factors and activation of anti-inflammatory transcription factors [12,13]. N-3 PUFAs may also decrease the production of pro-inflammatory n-6 PUFA derived lipid mediators and increase the production of anti-inflammatory mediators [12]. Both *de novo* synthesized and dietary fatty acids may affect inflammatory status. The former offers a potential approach to alter inflammatory status.

In EAT, fatty acid composition and gene expression related to intracellular fatty acid trafficking appear to differ by metabolic state [14,15]. This suggests a relation between EAT fatty acid metabolism and metabolic dysfunction. A recent clinical trial has demonstrated that the reduced risk of cardiovascular death by high-dose EPA supplementation (icosapent ethyl) is not completely explained by reductions in triglyceride concentrations or systemic inflammation [16]. This raises the integrating possibility that the intervention may in part have provided cardio-protective effects by influencing local inflammation. It has previously been found in humans that adipose tissue fatty acid composition relates to local adipose inflammation [17]. Hence, dietary interventions that alter EAT fatty acid composition could influence EAT inflammation and the development of CAD. The collection of EAT in humans typically occurs during elective cardiac procedures, a situation not conducive to prior dietary intervention. To our knowledge, no study has examined the effect of dietary patterns, differing in fat quality, on EAT fatty acid composition or its association with EAT inflammatory gene expression.

The Ossabaw miniature pig develops an EAT depot similar to humans [18]. We previously evaluated the Ossabaw miniature pig as a translational model to study dietary patterns and atherosclerosis [19]. Using this experimental model our objectives were to 1) to determine the influence of Western-type diet (WD; high in saturated fat) and Heart Healthy diet (HHD; high in unsaturated fat), with and without atorvastatin, on EAT fatty acid composition; and 2) to determine if proportions of select EAT SFAs and PUFAs relate to EAT inflammatory gene expression.

2. Materials and methods

2.1 Study design, animals, and diets

This study used samples generated from a previous investigation designed to determine the effect of dietary patterns and statin therapy on atherosclerosis development in the Ossabaw miniature pig [19]. Hence, the sample size was based on the original outcome of atherosclerosis. Research protocols and procedures have previously been reported [19] and were approved by the Beltsville Institutional Animal Care and Use Committee (IACUC). Additional approval for biological sample/tissue storage and analysis was obtained from the Tufts Medical Center/Tufts University IACUC. Thirty-two Ossabaw miniature pigs (16 boars, and 16 gilts, 5–8 weeks of age) were randomized into four groups using a 2×2 factorial design: WD, WD+statin (WD+S), and HHD+statin (HHD+S). Diets were designed to be similar to human dietary patterns and were fed in isocaloric amounts for 6 months following a 2-month acclimatization period. Both diets provided 38% energy from fat, 47% energy from carbohydrate and 15% energy from protein, and 2.5% wet weight (% w/w) of a vitamin and mineral mix [19]. Diets differed in the type and sources of dietary fat and carbohydrate (Fig. 1). The primary source of dietary fat in the WD was anhydrous milk fat (high in SFAs), while the primary sources in the HHD were canola, soybean and corn oils (high in MUFAs and PUFAs). The primary source of carbohydrate in the WD was refined, while the primary source in the HHD was whole grain. HHD-fed pigs were additionally fed a daily freeze-dried fruit and vegetable mix and supplemented with fish oil capsules (Epanova 1 g, 550 mg eicosapentaenoic acid [EPA, 20:5n-3]+200 mg docosahexaenoic acid [DHA, 22:6n-3], AstraZeneca, Wilmington, DE) 3 times per week. Diets differed in the amounts of fiber (HHD: 13 g/100 g, WD 7 g/100 g) and cholesterol (HHD: 0.1% w/w, WD: 1.5% w/w) [19]. The statin treated pigs received Atorvastatin (Lipitor, Pfizer, New York, NY) at 20 mg/day during months 1–3, and 40 mg/day during months 4–6 of the intervention period. Two pigs died during the course of the study; one in the WD group during the acclimatization period and one pig in the HHD group during the baseline blood draw, resulting in a final sample size of 30.

2.2 Sample collection

All samples were collected at the time of necropsy following euthanasia [19]. EAT adjacent to the proximal left anterior descending artery was collected, flash frozen in liquid nitrogen, and stored at –80°C until processing.

2.3 Measurement of EAT fatty acids

Lipids were extracted using a modified Folch procedure [20]. Individual fatty acids were separated by gas chromatography, identified using an authentic fatty acid internal standard (Nu-Chek Prep, Elysian, MN, USA), and expressed as molar percent (mol%) as described previously [21]. Thirty-six fatty acids were resolved. Fatty acid product-to-precursor ratios were calculated to estimate desaturase enzyme activities (stearoyl-CoA-desaturase 1 [SCD1; 16:1n-7/16:0], stearoyl-CoA-desaturase 2 [SCD2; 18:1n-9/18:0], delta-5-desaturase [D5D; 20:4n-6/20:3n-6]), and delta-6-desaturase [D6D; 20:3n-6/18:2n-6]) [22].

2.4 EAT gene expression

Gene expression of EAT homogenates was determined by RNA sequencing as described previously [23,24]. EAT RNA was isolated using RNeasy Universal Midi kit (Qiagen, Valencia, CA) per the manufacturer's instructions. RNA was treated with Turbo DNase (Ambion, Waltham, MA) and RNA quality was assessed using an Experion RNA analysis electrophoresis kit (Bio-Rad, Hercules, CA). Only samples with an RNA Quality Indicator (RQI) greater than 7 were sequenced. Samples were prepared for sequencing using the Illumina TruSeq RNA Sample Preparation Kit v2 (Illumina, San Diego, CA) and AMPure XP beads (Beckman Coulter, Brea, CA) per the manufacturer's instructions. DNA fragment size was determined using Experion DNA 1 K chips (Bio-Rad, Hercules, CA) and libraries were quantified using the KAPA Library Quantification kit (KAPA Biosystems, Wilmington, MA). Samples were sequenced using an Illumina NextSeq 500 sequencer (Illumina, San Diego, CA) with 100 base pair single end reads. Raw FASTQ data were processed for quality using CLC Bio Genomic Workbench (Qiagen, Valencia, CA). The EAT transcriptome was assembled using the annotated *Sus scrofa* 11.1-build as a reference genome [25]. Additional analysis was also performed using the Porcine Translational Research Database [26,27], as described below.

2.5 Statistical analysis

Data was analyzed using R (version 3.3.2) run on RStudio (version 1.0.153, RStudio: Integrated Development for R. RStudio, Boston, MA) and SAS for Windows (version 9.4; SAS Institute, Cary, NC). We removed data from one pig from the analysis due to sample oxidation that prevented the complete resolution of fatty acids. A two-way ANOVA (PROC GLM) with a Tukey-Kramer post hoc test was used to determine diet, statin, and diet × statin effects on EAT fatty acid composition. Residual plots were examined to determine normality and a Kruskal-Wallis test (PROC NPAR1WAY) was used for fatty acids not exhibiting a normal distribution. In all cases, the Kruskal-Wallis test and two-way ANOVA analyses were equivalent. Since results of the two-way ANOVA proved to be robust to departures from normality they are reported. A *P* .05 was considered statistically significant.

Spearman's correlation coefficients were calculated between the EAT fatty acids and EAT gene expression using pigs pooled from all groups (*n*=29). Gene expression data from prior RNA sequencing to determine the effect of the respective dietary patterns and statin therapy on the EAT transcriptome was used for a targeted analysis of EAT gene expression and fatty acid composition [23]. In the present study we selected genes of interest on an *a priori* basis

for their potential role in fatty acid mediated adipose tissue inflammation [28]. Genes were related to n-3 PUFA signaling (adiponectin [*ADIPOQ*], free fatty acid receptor 4 [*FFAR4*], and peroxisome proliferator activated receptor gamma [*PPARG*]), production of lipid-mediators (arachidonate–lipoxygenase [*ALOX5* and *ALOX15*] and prostaglandin-endoperoxide synthase 2 [*PTGS2*]), and inflammation (interleukin 1 beta [*IL-1 β*], toll like receptor 2 [*TLR2*], toll like receptor 4 [*TLR4*], interleukin 6 [*IL-6*], and monocyte chemotactic protein [*MCP1*]). We have previously published the independent effects of diet and statin therapy on EAT gene expression [23]. For the present study gene expression levels are summarized in Table A1. Gene expression of *IL-1 β* was only available from analysis using the Porcine Translational Database. The following fatty acids were selected based on their role in diet induced inflammation: SFAs (total SFAs, capric acid [10:0], lauric, acid [12:0]) palmitic acid [16:0], stearic acid [18:0]), n-6 PUFAs (total n-6 PUFAs, linoleic acid [18:2n-6], arachidonic acid [20:4n-6], and n-3 PUFAs (total n-3 PUFAs, α -linolenic acid, EPA, docosapentaenoic acid [DPA, 22:5n-3], and DHA). Bonferroni adjusted p-values were used to correct for multiple testing (13 fatty acids and 11 genes) and associations with an $r = +/-0.2$ and $P .0003$ were considered statistically significant. Additionally, Spearman's correlation coefficients were calculated to determine associations between estimated desaturase enzyme activities (SCD1, SCD2, D5D, and D6D) and expression of the aforementioned genes in EAT. Bonferroni adjusted p-values were used to correct for multiple testing (4 estimated desaturase activities and 11 genes) and we considered associations with an $r = +/-0.2$ and $P .001$ statistically significant. For all correlation analyses, values beyond 2 standard deviations of the mean were replaced with median proportion of the fatty acid for pigs in the respective diet +/- atorvastatin group.

In a secondary analysis we examined associations between EAT fatty acids and gene expression (as described above) using gene expression values based on alignment to the Porcine Translational Research Database in place of the *Sus scrofa* 11.1-build reference genome. The Porcine Translational Research Database is a manually curated database with annotation on over 10,000 genes with full length RNA transcripts [26]. Analysis with this manually curated database helps to ensure results are robust to potential limitations in annotation in the Ensembl genome that relies on machine-based annotation [27].

3. Results

3.1 EAT fatty acid composition

Overall, there was a significant effect of the diet on proportions of EAT SFAs, PUFAs, and *trans* fatty acids, but no significant effect of atorvastatin or significant diet \times statin interaction on EAT fatty acid composition (Table 1). Relative to the HHD, pigs fed the WD had significantly higher proportions of total SFAs and *trans* fatty acids; including palmitic acid, stearic acid, vaccenic acid (18:1n-7 t), and conjugated linoleic acid (18:2 CLA) (all $P < .01$). There were no significant differences in proportions of total MUFAs. However, pigs fed the HHD had higher proportions of oleic acid (18:1n-9), than WD fed pigs ($P < .01$). Pigs fed the HHD had higher proportions of total n-6 PUFAs and n-3 PUFAs; including linoleic acid, α -linolenic acid, EPA, and DPA (all $P < .01$) but not DHA. Estimated desaturase enzyme activity for SCD2 was higher in pigs fed the HHD, whereas estimated SCD1 and D6D

activities was higher in pigs fed the WD (all $P < .01$). Estimated D5D activity was similar between diets.

3.2 Associations between EAT fatty acids and gene expression

A heat map of summarizing correlation coefficients between selected SFAs and PUFAs (mol %), and gene expression (rpkm) is displayed in Fig. 2A, and the associated correlation coefficients in Table A2. Proportions of total and long-chain SFAs (palmitic and stearic acid) were positively associated with expression of *PTGS2* and *TLR2* (all $P < .0003$, Fig. 3). The medium-chain SFAs (capric and lauric acid) had inverse associations with expression of *ALOX15* and *IL-6* (all $P < .0003$). Proportions of all n-6 and n-3 PUFAs, except arachidonic acid, were positively associated with the expression of *PPARG* and *FFAR4* (all $P < .0003$, Fig. 4). With the exception of arachidonic acid and DHA, all other PUFAs were positively associated with expression of *ADIPOQ* (all $P < .0003$). Proportions of long-chain n-3 PUFAs (EPA, DPA, and DHA) were positively associated with *ALOX5* expression (all $P < .0003$). No statistically significant associations were identified between proportions of SFAs or PUFAs and expression of *TLR4* and *MCPI*.

3.3 Associations between EAT estimated desaturase activities and gene expression

Associations between EAT estimated desaturase enzyme activities and EAT gene expression are represented in Fig. 2B, all correlation coefficient values are included in Table A2. Estimated SCD2 and D5D activities were more commonly associated with gene expression. Estimated SCD2 activity was positively associated with the expression of genes involved in n-3 PUFA signaling (*PPARG*, *FFAR4*, and *ADIPOQ*), and *ALOX15* but were inversely associated with *PTGS2* and *TLR2* (all $P < .001$). In contrast, estimated D5D activity was inversely associated with the expression of genes involved in n-3 PUFA signaling (*PPARG*, *FFAR4*, and *ADIPOQ*) and, positively associated with *PTGS2*, *TLR2*, and *MCPI* (all $P < .001$, Fig. 5). There were no significant associations between estimated desaturase activities and *ALOX5*, *IL-1 β* , and *TLR4* expression.

3.4 Secondary analysis

Correlations between proportions of SFAs, PUFAs, and estimated desaturase activities with gene expression were similar using the Porcine Translational Research Database (Table A3). In this analysis, we additionally examined associated between SFAs, PUFAs and expression of *IL-1 β* and found no statistically significant associations (Table S3).

4. Discussion

Adipose tissue fatty acid composition may be related to adipose inflammation. However, little is known about the influence of dietary patterns, differing in fat type, and statin therapy on EAT fatty acid composition. We investigated the effect of dietary patterns differing in fatty acid profile fat on EAT fatty acid composition and the relationship between proportions of EAT fatty acids and inflammatory gene expression in the Ossabaw pig model. Our results indicate that dietary patterns, differing in dietary fat quality, are a significant determinant of EAT fatty acid composition and that proportions of SFAs and PUFAs associated with the expression of genes related to inflammation.

While EAT fatty acid composition was reflective of the respective dietary fat sources, we cannot rule out the possible contribution of endogenous synthesis and preferential storage of SFAs which accounted for a large proportion (38–48%) of total fatty acids across all groups of pigs [29]. Similarly, as MUFAs are endogenously synthesized and preferentially stored in adipose tissue [29], we observed no differences in the proportion of total MUFAs between diet groups. We did however note a significant diet effect on proportions of oleic acid, which may in part be due to the significant increase of estimated SCD2 activity, in pigs fed the HHD.

In general, proportions of EAT PUFAs were positively associated with the expression of genes involved in n-3 PUFA signaling (*FFAR4*, *PPARG* and *ADIPOQ*). N-3 PUFAs serve as ligands for both *PPARG* and *FFAR4* [30]. Following induction of *PPARG* downstream signaling may lead to increased expression of *ADIPOQ* and consequently the production of adiponectin; which is associated with reductions in adipose tissue inflammation and improvements insulin sensitivity [28]. Induction of *FFAR4* (also known as GPR120) by n-3 PUFAs inhibits inflammatory signaling [30,31]. Together these associations suggest dietary PUFAs may induce anti-inflammatory signaling in EAT.

PTGS2 and *ALOX* (*ALOX5* and *ALOX15*) genes are involved in the synthesis of lipid mediators derived from n-6 and n-3 PUFAs [32,33]. The n-6 PUFA arachidonic acid is considered the major substrate for these enzymes. When available, n-3 PUFAs can displace arachidonic acid in the cell membrane shifting the balance from arachidonic acid derived pro-inflammatory lipid mediators to n-3 PUFA derived less-inflammatory or anti-inflammatory mediators [32,33]. Positive associations between long-chain n-3 PUFAs (EPA, DPA, and DHA) and *ALOX5* expression support their role as both substrates and inducers of n-3 derived lipid mediators [34]. Notably, we did not observe associations between proportions of arachidonic acid and expression of any genes involved in lipid mediator synthesis.

Total and long-chain SFAs (palmitic and stearic) were positively correlated with *PTGS2*. This observation is consistent with prior work suggesting that SFAs induce *PTGS2* expression [35,36]. Total and long-chain SFAs were also associated with the expression of *TLR2*. SFA induced activation of *TLR* signaling evokes inflammatory signaling that contributes to adipose tissue inflammation [37]. We observed non-statistically significant trends towards a positive association between total SFAs and palmitic acid with *TLR4*. Unexpectedly, no significant associations were observed between any of the SFAs and expression of inflammatory cytokines *IL-6*, *IL-1 β* , and *MCPI*. This lack of significant associations between specific inflammatory genes and fatty acids may be due to greater than expected weight gain during the diet intervention period [19], independently resulting in induction of EAT inflammatory genes. Additionally, we measured gene expression in EAT homogenates which is reflective of multiple cells types. Hence, gene expression from cells responsible for production of inflammatory cytokines may have been diluted.

The estimated enzyme activity of D5D was more strongly associated with gene expression than other estimated enzyme activities. D5D facilitates the conversion of dihomo- γ -linolenic acid (20:3n-6) to arachidonic acid and eicosatetraenoic acid (20:4n-3) to EPA [38]. Hence,

the positive association between D5D and *PTGS2* could be reflective of higher substrate availability. Dietary PUFAs can inhibit desaturase (delta-5 and delta-6 desaturase) gene expression [39] which may explain inverse associations between estimated desaturase activity and genes involved in n-3 PUFA signaling (FFAR4, PPARG and ADIPOQ).

The original investigation was designed to compare the effects of two dietary patterns, a HHD and WD, on the development of atherosclerosis in Ossabaw miniature pigs, rather than dietary fat type, *per se* [19]. This approach increases the translational value of our study. However, we cannot rule out the possibility that other dietary components influenced EAT fatty acid composition and gene expression. While differences in human and porcine fatty acid metabolism exist our results are in agreement with prior work suggesting the preferential storage of oleic and palmitic acid in human EAT [40]. One key mechanism by which PUFAs may modulate inflammation is via the production of lipid mediators. Though our results suggest a shift in balance from arachidonic acid to n-3 PUFAs derived lipid mediators with the HHD, we cannot confirm this without a direct measurement. Additionally, the only adipose tissue depot assessed was EAT. Future work comparing additional adipose tissue depots will help determine unique influences of diet on EAT. Difficulties associated with porcine research make it challenging to achieve a large sample size. However, these models allow for comprehensive assessment of the interplay between diet and tissues that are challenging to investigate in humans.

We conclude that HHD and WD, differing in dietary fat quality, have differential influences on EAT fatty acid composition and subsequent gene expression. Associations between EAT SFAs with genes involved in inflammatory signaling, and EAT PUFAs with genes involved in anti-inflammatory signaling, provide a link between diet and EAT inflammation. Targeted changes in dietary quality may be a viable nutritional strategy to reduce EAT inflammation and in turn effect the development of CAD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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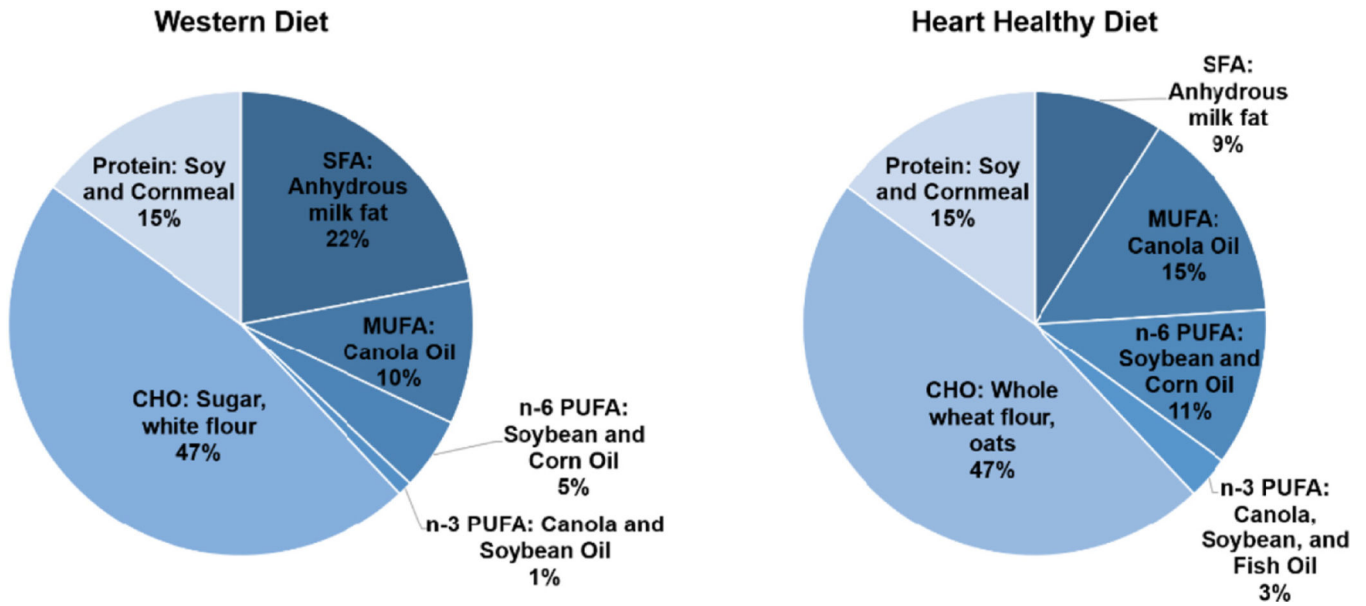


Fig. 1. Macronutrient composition (% energy) and food sources of the Western and Heart Healthy diets. Macronutrient composition was confirmed by chemical analysis (Cumberland Valley Analytical Services, Hagerstown, MD, USA, and Eurofins Scientific, Des Moines, IA, USA). CHO: carbohydrate, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

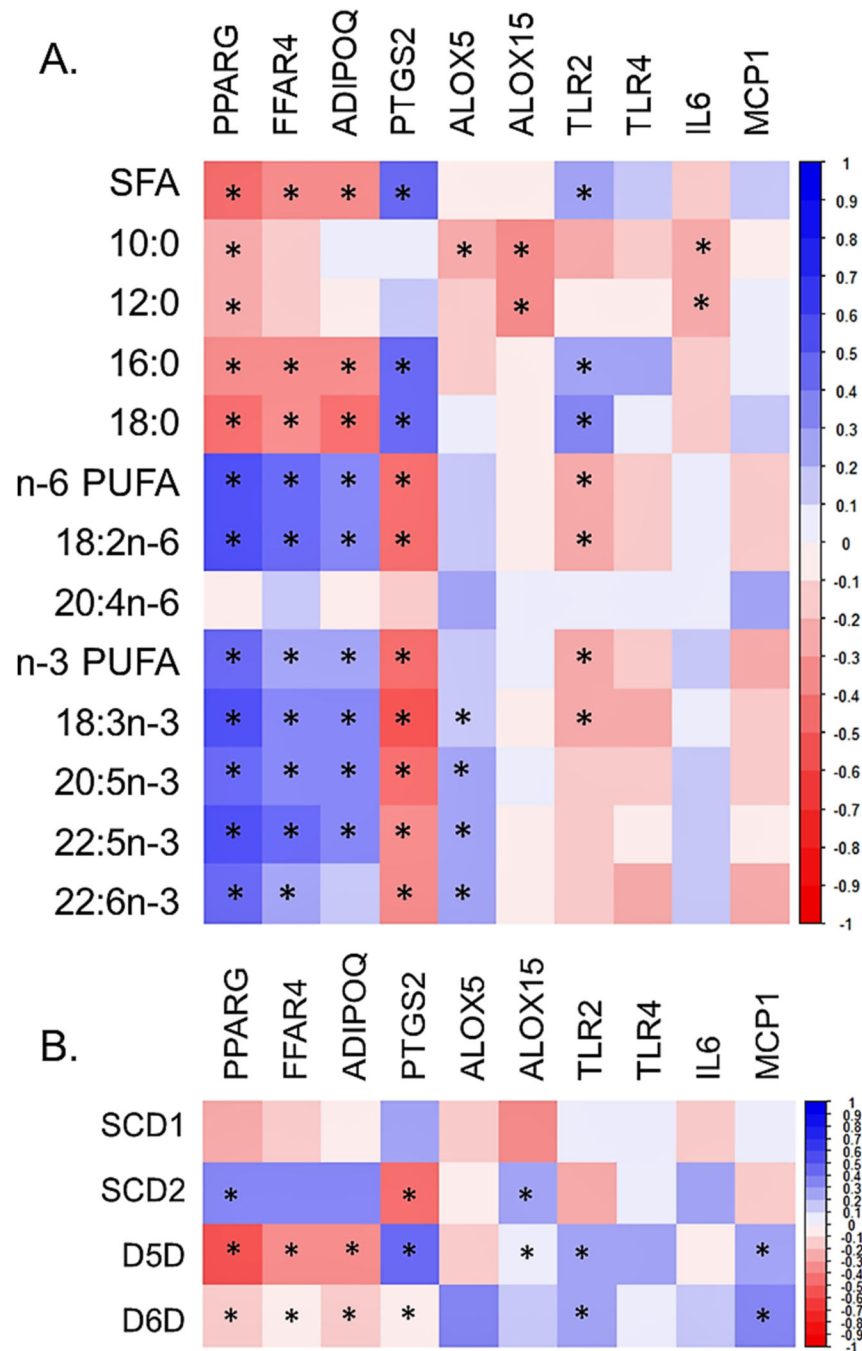


Fig. 2. (A) Heat map representing Spearman's correlation coefficients between proportions (mol%) of SFAs, capric acid, lauric acid, palmitic acid, stearic acid, PUFAs n-6, linoleic acid, arachidonic acid, n-3 PUFAs, α -linolenic acid, EPA, DPA, and DHA and EAT gene expression (rpkm). A Bonferroni correction was used to adjust for multiple comparisons ($*P$.0003). (B) Heat map representing Spearman's correlation coefficients between EAT estimated desaturase enzyme activities and EAT gene expression (rpkm). A Bonferroni correction was used to adjust for multiple comparisons ($*P$.001).

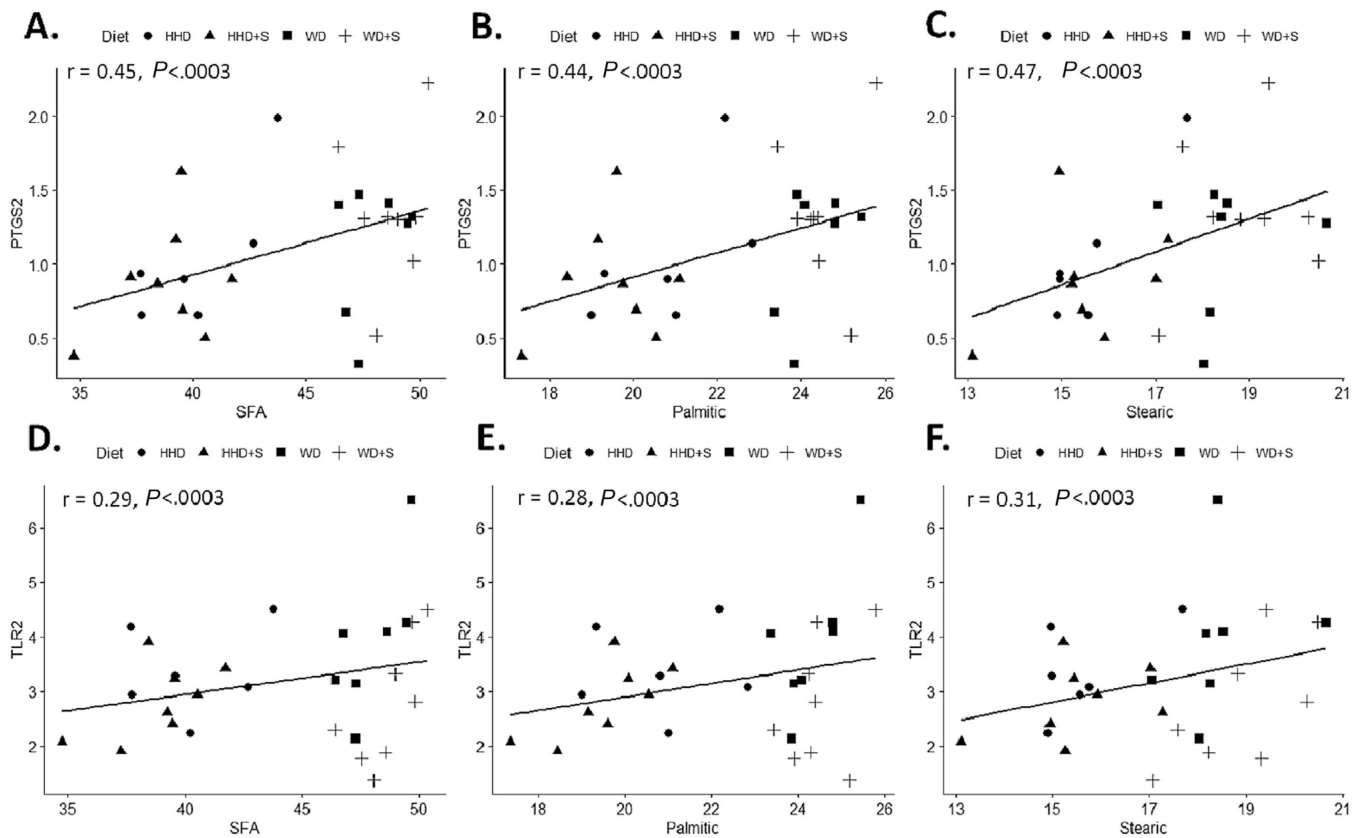


Fig. 3.

Scatter plots displaying the correlations between (A) the proportion of total SFA (mol %) and *PTGS2* gene expression (rpkm) in EAT. (B) the proportion of palmitic acid (mol %) and *PTGS2* gene expression (rpkm) in EAT. (C) the proportion of stearic acid (mol %) and *PTGS2* gene expression (rpkm) in EAT. (D) the proportion of total SFA (mol %) and *TLR2* gene expression (rpkm) in EAT. (E) the proportion of palmitic acid (mol %) and *TLR2* gene expression (rpkm) in EAT. (F) the proportion of stearic acid (mol %) and *TLR2* gene expression (rpkm) in EAT. HHD: Heart Healthy diet, WD: Western diet, WD+S: Western diet+atorvastatin, HHD+S: heart healthy diet+atorvastatin, *PTGS2*: prostaglandin-endoperoxide synthase 2, SFA: saturated fatty acids, *TLR2*: toll like receptor 2, EAT: epicardial adipose tissue. All correlations were statistically significant ($P < .0003$).

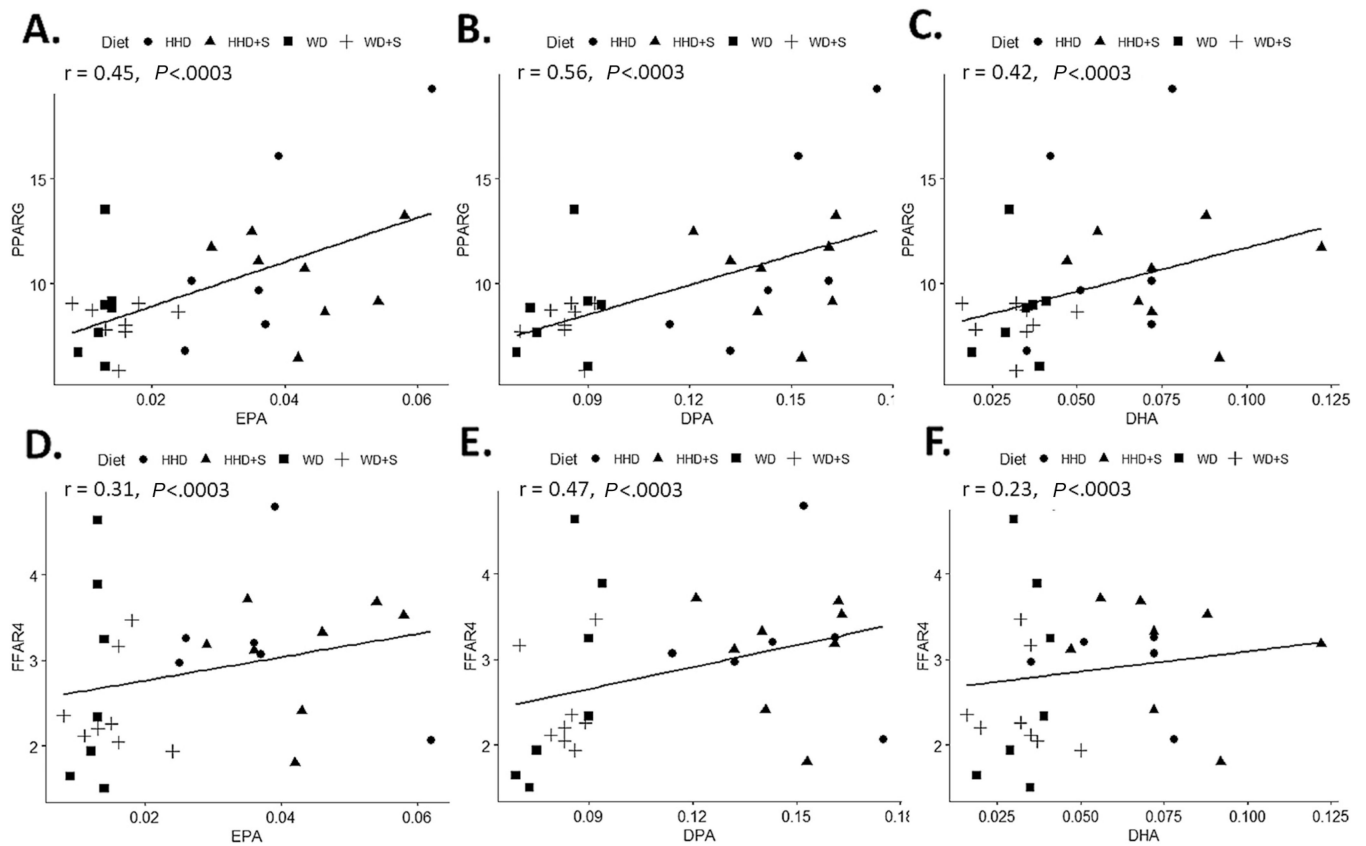


Fig. 4.

Scatter plots displaying the correlations between (A) the proportion of EPA (mol %) and *PPARG* gene expression (rpkms) in EAT. (B) the proportion of DPA (mol %) and *PPARG* gene expression (rpkms) in EAT. (C) the proportion of DHA (mol %) and *PPARG* gene expression (rpkms) in EAT. (D) the proportion of EPA (mol %) and *FFAR4* gene expression (rpkms) in EAT. (E) the proportion of DPA (mol %) and *FFAR4* gene expression (rpkms) in EAT. (F) the proportion of DHA (mol %) and *FFAR4* gene expression (rpkms) in EAT. HHD: Heart Healthy diet, WD: Western diet, WD+S: Western diet+atorvastatin, HHD+S: heart healthy diet+atorvastatin, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid *FFAR4*: free fatty acid receptor 4, *PPARG*: peroxisome proliferator activated receptor, EAT: epicardial adipose tissue. All correlations were statistically significant ($P < .0003$).

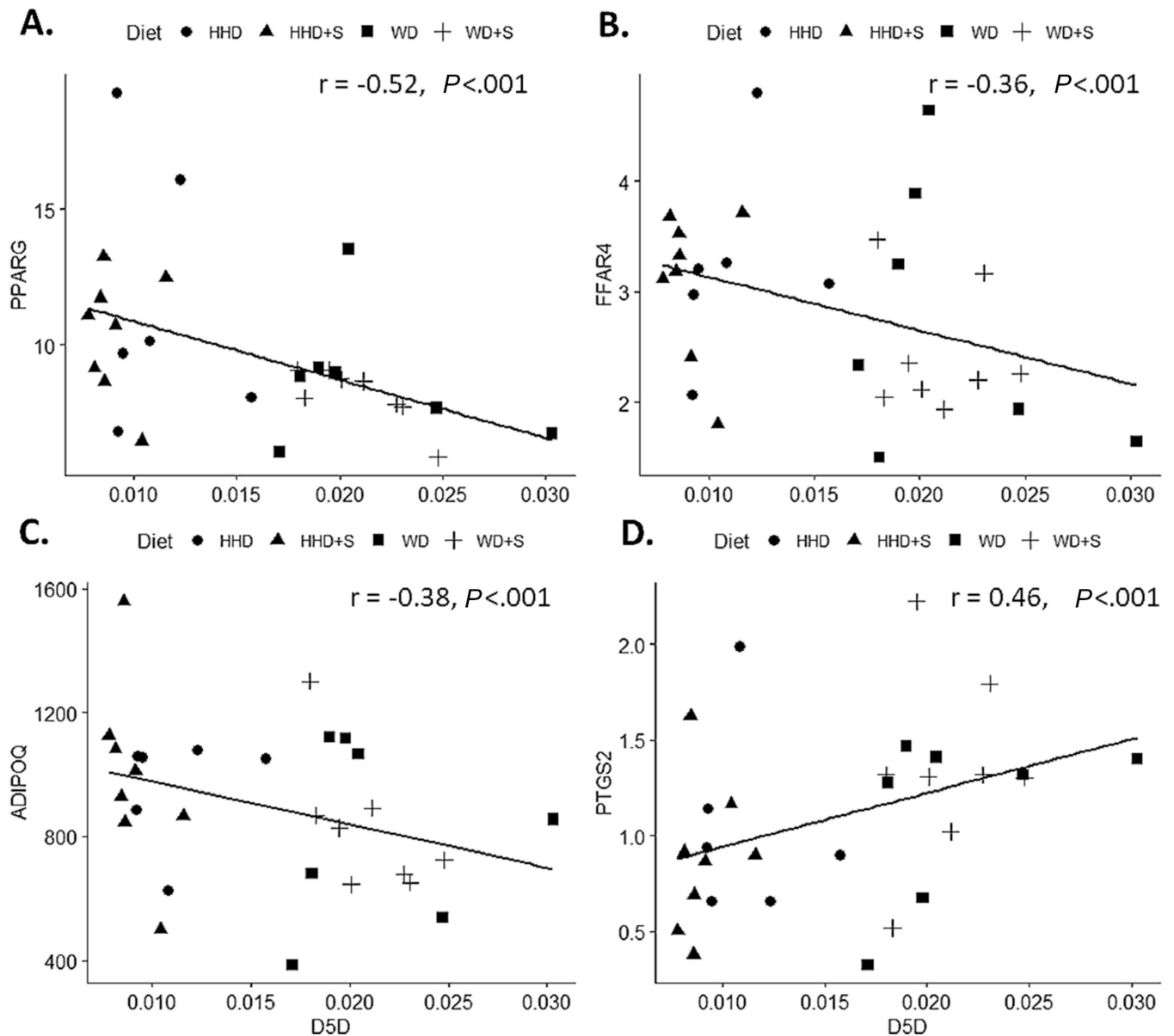


Fig. 5. Scatterplots displaying the correlations between (A) estimated D5D activity and *PPARG* gene expression (rpkm) in EAT. (B) estimated D5D activity and *FFAR4* gene expression (rpkm) in EAT (C) estimated D5D activity and *ADIPOQ* gene expression (rpkm) in EAT (D) estimated D5D activity and *PTGS2* gene expression (rpkm) in EAT. HHD: Heart Healthy diet, WD: Western diet, WD+S: Western diet+atorvastatin, HHD+S: heart healthy diet +atorvastatin, D5D: delta-5-desaturase (20:4n-6/20:3n-6), *PPARG*: peroxisome proliferator activated receptor gamma, *FFAR4*: free fatty acid receptor 4, *ADIPOQ*: adiponectin, *PTGS2*: prostaglandin-endoperoxide synthase 2, EAT: epicardial adipose tissue. All correlations were statistically significant ($P < .001$).

Table 1

EAT fatty acid composition (mean \pm SD) of Ossabaw miniature pigs fed a HHD or WD +/- atorvastatin at the conclusion of the study¹

Fatty acid (mol%)	P value							
	WD	WD+S	HHD	HHD+S	Diet	Statin	Diet \times Statin	
SFAs	47.95 \pm 1.31 ^a	48.69 \pm 1.32 ^a	40.27 \pm 2.51 ^b	38.87 \pm 2.13 ^b	<.01	.68	.13	
10:0	0.16 \pm 0.04 ^a	0.17 \pm 0.05 ^a	0.10 \pm 0.01 ^b	0.10 \pm 0.02 ^b	<.01	.58	.88	
12:0	0.27 \pm 0.05 ^a	0.30 \pm 0.06 ^a	0.14 \pm 0.02 ^b	0.15 \pm 0.01 ^b	<.01	.31	.49	
14:0	4.00 \pm 0.47 ^a	4.12 \pm 0.54 ^a	2.91 \pm 0.40 ^b	2.86 \pm 0.42 ^b	<.01	.85	.65	
15:0	0.46 \pm 0.07 ^a	0.45 \pm 0.08 ^a	0.32 \pm 0.05 ^b	0.32 \pm 0.05 ^b	<.01	.84	.90	
16:0	24.32 \pm 0.72 ^a	24.45 \pm 0.73 ^a	20.85 \pm 1.52 ^b	19.49 \pm 1.19 ^b	<.01	.16	.07	
18:0	18.44 \pm 1.09 ^a	18.89 \pm 1.21 ^a	15.63 \pm 1.06 ^b	15.52 \pm 1.3 ^b	<.01	.69	.53	
20:0	0.25 \pm 0.02	0.25 \pm 0.06	0.28 \pm 0.04	0.27 \pm 0.04	.14	.71	.93	
22:0	0.03 \pm 0.00	0.05 \pm 0.06	0.03 \pm 0.00	0.15 \pm 0.33	.40	.31	.49	
24:0	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.01	0.01 \pm 0.01	.04	.80	1.00	
MUFAs	43.34 \pm 2.22	42.52 \pm 1.76	44.37 \pm 1.84	43.60 \pm 1.61	.15	.26	.97	
14:1n-5	0.10 \pm 0.02 ^a	0.11 \pm 0.03 ^a	0.03 \pm 0.01 ^b	0.03 \pm 0.01 ^b	<.01	.37	.56	
16:1n-9	0.39 \pm 0.05	0.39 \pm 0.05	0.33 \pm 0.07	0.38 \pm 0.05	.14	.16	.19	
16:1n-7	2.48 \pm 0.17 ^a	2.45 \pm 0.21 ^a	1.45 \pm 0.12 ^b	1.39 \pm 0.18 ^b	<.01	.49	.78	
18:1n-9	36.83 \pm 2.01 ^b	36.27 \pm 1.81 ^b	38.97 \pm 1.89 ³	38.24 \pm 1.34 ^a	<.01	.34	.90	
18:1n-7	2.70 \pm 0.20 ^a	2.55 \pm 0.11 ^a	2.36 \pm 0.09 ^b	2.42 \pm 0.18 ^b	<.01	.39	.09	
20:1n-9	0.77 \pm 0.14 ^b	0.67 \pm 0.09 ^b	0.96 \pm 0.17 ^a	0.87 \pm 0.14 ^a	<.01	.08	.87	
22:1n-9	0.07 \pm 0.02 ^b	0.07 \pm 0.01 ^b	0.26 \pm 0.03 ^a	0.25 \pm 0.04 ^a	<.01	.80	.82	
24:1n-9	0.00 \pm 0.00	0.01 \pm 0.02	0.01 \pm 0.00	0.01 \pm 0.00	.45	.49	.29	
n-6 PUFAs	6.86 \pm 1.58 ^b	6.88 \pm 1.15 ^b	12.63 \pm 2.55 ^a	14.82 \pm 2.86 ^a	<.01	.20	.19	
18:2n-6	6.05 \pm 1.51 ^b	6.09 \pm 1.13 ^b	11.55 \pm 2.43 ^a	13.69 \pm 2.76 ^a	<.01	.18	.18	
18:3n-6	0.03 \pm 0.01	0.03 \pm 0.01	0.04 \pm 0.01	0.04 \pm 0.02	.02	.57	.56	
20:2n-6	0.28 \pm 0.06 ^b	0.26 \pm 0.04 ^b	0.56 \pm 0.09 ^a	0.58 \pm 0.09 ^a	<.01	.93	.44	
20:3n-6	0.12 \pm 0.01	0.13 \pm 0.01	0.13 \pm 0.02	0.12 \pm 0.02	.86	.91	.68	
20:4n-6	0.27 \pm 0.03	0.27 \pm 0.05	0.26 \pm 0.06	0.28 \pm 0.05	.81	.47	.70	

Fatty acid (mol%)	P value						
	WD	WD+S	HHD	HHD+S	Diet	Statin	Diet × Statin
22:4n-6	0.09±0.01	0.09±0.01	0.09±0.01	0.09±0.01	.19	.53	.95
22:5n-6	0.02±0.01 ^a	0.01±0.00 ^a	0.01±0.00 ^b	0.01±0.00 ^b	.02	.80	.05
n-3 PUFAs	0.56±0.17 ^b	0.59±0.08 ^b	2.32±0.76 ^a	2.27±0.43 ^a	<.01	.94	.82
18:3n-3	0.37±0.13 ^b	0.4±0.08 ^b	1.7±0.36 ^a	1.98±0.41 ^a	<.01	.18	.24
18:4n-3	0.03±0.01 ^a	0.04±0.01 ^a	0.02±0.01 ^b	0.02±0.00 ^b	<.01	.63	.58
20:5n-3	0.01±0.00 ^b	0.02±0.00 ^b	0.04±0.01 ^a	0.04±0.01 ^a	<.01	.22	.65
22:5n-3	0.08±0.01 ^b	0.08±0.01 ^b	0.15±0.02 ^a	0.15±0.02 ^a	<.01	.89	.96
22:6n-3	0.06±0.08	0.05±0.06	0.09±0.08	0.08±0.02	.34	.72	.97
Trans	1.29±0.17 ^a	1.33±0.18 ^a	0.42±0.06 ^b	0.45±0.05 ^b	<.01	.51	.92
16:1n-7t	0.03±0.02 ^a	0.03±0.02 ^a	0.01±0.01 ^b	0.02±0.01 ^b	<.01	.62	.96
16:1n-9t	0.14±0.01 ^a	0.15±0.01 ^a	0.04±0.00 ^b	0.04±0.00 ^b	<.01	.42	.58
18:1n-7t	0.22±0.06 ^a	0.23±0.05 ^a	0.06±0.01 ^b	0.07±0.02 ^b	<.01	.15	.58
18:1n-9t	0.20±0.04 ^a	0.22±0.04 ^a	0.07±0.02 ^b	0.08±0.01 ^b	<.01	.53	.83
18:1n-10-12t	0.27±0.02 ^a	0.28±0.03 ^a	0.12±0.01 ^b	0.12±0.01 ^b	<.01	.50	.48
18:2t	0.14±0.01 ^a	0.15±0.02 ^a	0.04±0.01 ^b	0.04±0.01 ^b	<.01	.97	.85
18:2CLA	0.28±0.03 ^a	0.27±0.04 ^a	0.09±0.02 ^b	0.09±0.01 ^b	<.01	.81	.71
Ratios							
SCD1	0.10±0.01 ^a	0.10±0.01 ^a	0.07±0.00 ^b	0.07±0.01 ^b	<.01	.90	.56
SCD2	2.01±0.20 ^b	1.93±0.16 ^b	2.50±0.24 ^a	2.48±0.17 ^a	<.01	.45	.73
D5D	2.17±0.32	2.19±0.43	2.09±0.22	2.32±0.28	.74	.34	.40
D6D	0.02±0.00 ^a	0.02±0.00 ^a	0.01±0.00 ^b	0.01±0.00 ^b	<.01	.29	.46

^aValues presented as mean ± SD. Superscripts denote a significant diet effect determined by a two-way ANOVA in which means without a common superscript differ ($p < .05$). EAT: epicardial adipose tissue, WD: Western diet, HHD: heart healthy diet, WD+S: Western diet+atorvastatin, HHD+S: heart healthy diet+atorvastatin, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Trans: trans fatty acids, CLA: conjugated linolenic acid, SCD1: stearoyl-CoA-desaturase 1 (16:1n-7/16:0), SCD2: stearoyl-CoA-desaturase 2 (18:1n-9/18:0), D5D: delta-5-desaturase (20:4n-6/20:3n-6), D6D: delta-6-desaturase (20:3n-6/18:2n-6).