SUPPLEMENTAL MATERIALS & METHODS

Feeding protocols - Feeding studies were repeated twice and conducted over two different time periods. A total of 150 nine-week-old male LDLR-/- mice were purchased from the Jackson Laboratory. In each set of studies, 75 mice were randomly assigned to five groups of 15 animals each after 1 week of acclimatization. LDLR-/- mice were fed custom-made eucaloric diets manufactured by Harlan Teklad; a low-fat chow diet or high-fat, high-cholesterol diet enriched in either saturated fats (coconut oil), n-3 fats (menhaden oil), or a combination of SAT and n-3 fats in ratios of 3:1 (25% n-3) or 1:1 (50% n-3) for 12 weeks. The high-fat diets contained 185 g fat and 2 g cholesterol per kg of diet and varied only in lipid composition. The low-fat chow diet contained 50 g fat and 0.2 g cholesterol per kg of diet. Supplemental Tables I and II list the detailed composition of macronutrients and measured fatty acid profile of each diet, respectively. The food consumption of mice was monitored daily, and their body weight was measured weekly. All experimental procedures were approved by the Columbia University Institutional Animal Care Committee.

Plasma lipid analyses - Fasting mouse plasma was collected and measured for levels of free FA (FFA) (NEFA C kit, Wako), TG, and total cholesterol (Chol) (Roche Diagnostics) according to the manufacturer's procedures as previously detailed [1]. Blood was collected by retro-orbital venous plexus puncture of anesthetized mice at 4- and 8-wk and by cardiac puncture at the end of the 12-wk feeding periods [2].

Fatty acid composition of mouse feed and aorta - FA analysis was performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flamed ionization detector (GLC). Lipid extracts from mouse plasma, artery and mouse feed from each feeding group were chromatographed as methyl esters on a 30 m fused silica column with an internal diameter of 0.25 mm. The column was wall-coated with 0.2 μm SP-2380. Helium was used as carrier gas. The split ratio was 17:1. The injector and detector temperatures were 200˚C and 250˚C, respectively. The column temperature was held at 80˚C for 5 min and in a step-wise fashion then reached a plateau of 220˚C. The gas chromatograph was calibrated using a standard mixture

of FA (Sigma-Aldrich). A correction factor was applied to compensate for the lower ionization detector response to unsaturated FA relative to corresponding weights of saturated FA. Individual fatty acid methyl esters were calculated using the area corresponding to the C 15:0 internal standard. Results were normalized for weight percent of each FA [3, 4].

Immunofluorescent studies - The localization of arterial LpL with aortic cells was examined by immunofluorescence (IFC) similar to the process described previously [2]. At the end of the feeding period, mice were euthanized and perfused extensively. Aortas were dissected from the middle of left ventricle to the iliac bifurcation using a stereoscopic zoom microscope. Dissected aortas were fixed in 4% paraformaldehyde on ice for 2 h followed by the equilibration in 30% sucrose solution at 4°C overnight. The equilibrated samples were cut proximal to the descending aorta, and the proximal samples at the levels of the aortic valve (aortic root), ascending aorta, aortic arch the beginning of the descending aorta were positioned vertically and embedded in Tissue-Tek OCT (Sakura Finetek). Embedded samples were first frozen on dry ice and then stored in a -80°C freezer. Embedded samples were sectioned with a cryomicrotome. Sections of proximal aorta and aortic root were co-incubated with primary goat anti-LpL, rat anti-EC (CD31) and rabbit anti-macrophage (CD68) antibodies for 24 h at 4°C, followed by 1-h incubation of the mixture of corresponding secondary antibodies of Alexa 647 anti-goat IgG, Alexa 488 anti-rat IgG, and Alexa 546 anti-rabbit IgG. Fluorescence in the arterial wall was analyzed using a laser scanning confocal microscope (LSM-510 META). An argon laser (488 nm) and two helium-neon lasers (543 nm and 633 nm) were utilized for the excitation of Alexa 488, 546, and 647, respectively. Fluorescence of LpL and macrophages was quantitated as previously detailed [2, 5]. Results from at least six different sections of the same proximal aorta per mouse were averaged in each group (n=3-4)

Laser capture microdissection (LCM) - Macrophages from the aortic root were extracted by LCM for LpL and PPARs expression. Macrophages were detected on slides using an adapted rapid staining protocol [6]. Slides stained for macrophages were used to navigate and select macrophage-containing areas on the adjacent slides. Sequential slides with corresponding area of macrophages were stained with Harris

modified hematoxylin and eosin and were used for microdissection. Laser capture was performed using PALM MicroBeam IV (P.A.L.M. Microlaser Technologies) with a 355-nm solid-state ultraviolet (UV) laser. PALM RoboSoftware was used for the control of microdissection, laser function and contact-free transport. In brief, microdissection was performed by cutting around the perimeters of the selected area with a fine-focused laser beam. After cells were isolated, pulses of the focused laser beam just below the focal plane of the tissue specimen were used to create a pressure wave separating the targeted tissue from the slide and catapulting it against gravity into the microcentrifuge cap containing RNA lysis buffer. RNA extraction, amplification and analyses from collected aortic macrophages were performed following the manufacturer's instructions (MicroRNA Isolation kit, Stratagene, and MessageAmp II aRNA Amplification Kit, Ambion).

Quantitative Real-time PCR - Total RNA was isolated from mouse proximal aorta with TRIzol reagent (Invitrogen). Single strand cDNA was synthesized from RNA using iScript reverse transcriptase according to the manufacturer's instructions (Invitrogen). Quantitative real-time PCR was carried out on an iCycler (Bio-Rad) using SYBR PCR kit (Applied Biosystems). Results were analyzed by comparing the threshold crossing (*Ct*) of each sample after normalization to control genes (ΔCt). Changes in the threshold crossing (ΔCt) were used to calculate relative levels of each mRNA using the formula $2^{-\Delta Ct}$. All real-time PCR reactions were carried out at least twice from independent cDNA preparations. Supplemental Table III lists the primer sequences.

Morphometric analyses of atherosclerosis - Atherosclerotic lesion development was measured at the aortic root. Aortic samples starting at the aortic valve were collected as described above. Nine-μm thick sections of aortic valve were collected when the presence of aortic sinus was reached until the length of aorta sectioned was ~ 900 μm. We placed the sections sequentially onto six slides. Each of the six slides had six step sections that were 54 μm apart over a 324-μm area. Every first and seventh slides from each mouse was stained with Harris modified hematoxylin and Oil-Red-O (Fisher Scientific) [7]. Quantitative analyses of the atherosclerotic lesion development at aortic valve were measured using ImagePro imaging

software and Photoshop. In the separate set of studies, serial cross sections (5 μm) were collected and quantitated for lesion development using Image-Pro Plus software. Aortic lesion formation in each animal was measured as total lesion area (μm^2) per section from the point at which all three aortic valve leaflets first appeared according to the method by Paigen *et al.*[7].

Statistical analyses- Student's *t*-tests of group means were used to compare endpoints, and ANOVA was used to evaluate potential interactions between diets. The results from two sets of feeding studies were combined by normalizing to the mean of each chow-fed group and are expressed as the mean fold change±SE. Statistical significance was determined at the level of $p<0.05$.

REFERENCES

1. Qi K, Seo T, Al-Haideri M, Worgall TS, Vogel T, Carpentier YA, Deckelbaum RJ. Omega-3 triglycerides modify blood clearance and tissue targeting pathways of lipid emulsions. *Biochemistry*. 2002;41:3119-3127.
2. Chang CL, Seo T, Du CB, Accili D, Deckelbaum RJ. n-3 Fatty acids decrease arterial low-density lipoprotein cholesterol delivery and lipoprotein lipase levels in insulin-resistant mice. *Arterioscler Thromb Vasc Biol.* 2010;30:2510-2517.
3. Oliveira FL, Rumsey SC, Schlotzer E, Hansen I, Carpentier YA, Deckelbaum RJ. Triglyceride hydrolysis of soy oil vs fish oil emulsions. *JPEN J Parenter Enteral Nutr.* 1997;21:224-229.
4. Lepage G, Roy CC. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J Lipid Res.* 1984;25:1391-1396.

5. Swayne TC, Boldogh IR, Pon LA. Imaging of the cytoskeleton and mitochondria in fixed budding yeast cells. *Methods Mol Biol.* 2009;586:171-184.

6. Trogan E, Choudhury RP, Dansky HM, Rong JX, Breslow JL, Fisher EA. Laser capture microdissection analysis of gene expression in macrophages from atherosclerotic lesions of apolipoprotein E-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 2002;99:2234-2239.

7. Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA. Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis.* 1987;68:231-240.

Supplemental Table I. Macronutrient composition of diets *.

Ingredients (g/Kg)	**Chow**	**SAT**	**S:n-3 3:1****	**S:n-3 1:1****	**n-3**
Casein	200	200	200	200	200
L-Cystine	3	3	3	3	3
Corn-Starch	413	110	110	110	110
Maltodextrine	100	100	100	100	100
Sucrose	180	180	180	180	180
Soybean Oil	50				
Coconut Oil		144.35	108.26	72.18	
Olive Oil		23.17	17.38	11.58	
Corn Oil		16.95	16.95	16.95	16.95
Fish oil			41.88	83.76	168
Cholesterol	0.2	2	2	2	2

*All diets contained 20% protein (w/w), and were supplemented with vitamins and minerals to meet National Research Council requirements.
** S:n-3 3:1 and S:n-3 1:1 diets contained saturated FA-rich coconut oil and n-3 FA-rich fish oil at the ratios of 3:1 and 1:1, respectively.

Supplemental Table II. Fatty acid composition of diets .

% of total FA	Chow	SAT	S:n-3 3:1	S:n-3 1:1	n-3
12:0	0.0	43.0	32.5	21.8	0.0
14:0	0.5	15.6	13.9	12.3	8.9
16:0	12.8	10.7	12.3	14.0	17.5
16:1	0.0	0.2	3.2	6.2	12.3
18:0	4.6	2.8	2.9	3.0	3.1
18:1	21.9	18.7	17.3	15.9	13.0
18:2	53.2	8.8	8.6	8.4	8.0
18:3	6.6	0.2	0.6	1.1	2.0
20:3	0.2	0.0	0.0	0.0	0.0
20:4	0.2	0.0	0.6	1.2	2.5
20:5	0.0	0.0	4.2	8.4	17.0
22:5	0.0	0.0	1.0	2.1	4.2
22:6	0.0	0.0	2.8	5.6	11.5

Fatty acid composition of mouse feed was measured by GLC as described in Methods. Results are the mean of analyses of 2 samples and are very similar to the values that were calculated from ingredient analysis and manufacturer's data (Harlan-Teklad).

Supplemental Table III. Primer sequences

Primer	Forward Primer	Reverse Primer
CD68	CTTCCCACAGGCAGCACAG	AATGATGAGAGGCAGCAAGAGG
IL-6	AACCACGGCCTTCCCTACTTC	GCCATTGCACAACTCTTTTCTCAT
VCAM-1	CCCAAGGATCCAGAGATTCA	TAAGGTGAGGATGGCATTTC
IL-0	ATTTGAATTCCCTGGGTGAGAAG	CACAGGGGAGAAATCGATGACA
LpL	GAGGAATCTAATGGCCCATAGC	GGAACTCACTCTGTAAACCAGG
PPARα	CCTCTTCCCAAAGCTTGACAAGT	ATTAAGTTACAACAGTCAGTCCAAGCA
PPARγ	TCTCTCCGTAATGGAAGACC	GCATTATGAGACATCCCCAC
PPARβ/δ	AGATGGTGGCAGAGCTATGACC	TCTCCTCCTGTGGCTGTTCC
GAPDH	TGCAGTGGCAAAGTGGAGAT	TTGAATTTGCCGTGAGTGGA

Supplemental Figure I

Effects of diet on body weight in LDLR-/- mice. Each point represents the mean±SE of 15 mice. *, $p<0.01$. SAT/S:n-3 3:1 vs. chow; #, $p<0.01$, SAT/ S:n-3 3:1 vs. n-3.