

## **Supplemental Material**

### **Measurement of 8-HEPE in plasma**

Blood plasma was mixed with acetonitrile in 1M acetic acid (ratio, 1:3). The samples were centrifuged and the supernatants collected. The supernatants were concentrated on a MonoSpin C18 column (GL Science Inc.). The extraction efficiency is  $92 \pm 4.3$  %. 8-HEPE and EPA were separated on an InertSustain ODS-3 column (2.0 mm dia.  $\times$  250 mm; GL Science Inc.) with gradient elution (acetonitrile/water/formic acid, 30/70/0.1 to 90/10/0.1 in 30 min) at a flow rate of 0.2 mL/min. The compounds were identified and quantified by liquid chromatography-time of flight mass spectrometry (LC-TOFMS) (Agilent Technologies) using Agilent Mass Hunter Workstation Software (Agilent Technologies).

Supplemental Table 1

	HFD-60 (High-fat diet)	AIN-93M (Low-fat diet)
	(g / kg diet)	
Casein	200	140
L-cystine	3.6	1.8
Mart-Dextrin	60	-
Cornstarch	-	465.692
Alfarized cornstarch	160	155
Sucrose	55	100
Soybean oil	20	40
Lard	330	-
Cellulose	66.1	50
AIN-93 mineral mixture	35	35
AIN-93 vitamin mixture	10	10
Calcium carbonate	1.8	-
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	-	0.008

Supplemental Table 1. Compositions of the diets.

Supplemental Table 2

Symbol	Organism	Description	Ref seq DNA ID	Real-time PCR primers
<i>Actb</i>	Mus musculus	beta actin	NM_007393	TGGAATCCTGTGGCATCCATGAAAC
				TAAAACGCAGCTCAGTAACAGTCCG
<i>Fabp1</i>	Mus musculus	Fatty acid binding protein 1, liver	NM_017399	GTGGTCCGCAATGAGTTCAC
				CACCTTCCAGCTTGACGACT
<i>Cyp4a10</i>	Mus musculus	cytochrome P450, family 4, subfamily a, polypeptide 10	NM_010011	TTCCAGCAGTTCCCATCACC
				CTTGCTTCCCAGAACCATCT
<i>Ehhadh</i>	Mus musculus	Enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	NM_023737	GGCTAGAGCCCTGCAGTACGC
				CGATGCCTCGGCCATCGTTC
<i>Cpt1a</i>	Mus musculus	Carnitine palmitoyltransferase 1a, liver	NM_013495	TGGACCCCTCCCTGGGCATG
				ATCGCCACCCAGAGCCCTGT
<i>Cpt2</i>	Mus musculus	Carnitine palmitoyltransferase 2	NM_009949	CAGTGTGGGCGAGCTTCAGC
				TCAGGCAGGGTGACCCCTCT
<i>Fabp4</i>	Mus musculus	Fatty acid binding protein 4, adipocyte	NM_024406	GATGCCTTTGTGGGAACCT
				CTGTGCTCTGCGGTGATTT
<i>Pparg</i>	Mus musculus	Peroxisome proliferator activated receptor gamma	NM_0011273301	TGCCTTCGCTGATGCACTGCC
				CACGGAGAGGTCCACAGAGCTGA
<i>Cepba</i>	Mus musculus	CCAAT/enhancer binding protein, alpha	NM_007678	CCAACCCCATCCCCAACGGC
				GAAGCGGTCCAGCCCTGCTC
<i>Adipoq</i>	Mus musculus	Adiponectin	NM_009605	GCACTGGCAAGTTCTACTGCAA
				GTAGGTGAAGAGAACGGCCTTGT
<i>Srebf1</i>	Mus musculus	Sterol regulatory element binding transcription factor 1	NM_011480	GGACACAGCGGTTTTGAACG
				CTCAGGAGAGTTGGCACCTG
<i>Fasn</i>	Mus musculus	Fatty acid synthase	NM_007988	ATCTCTCCAAGTTCGACGCC
				GTTCTGTTCTCGGAGTGAGG
<i>Lpin2</i>	Mus musculus	Lipin 2	NM_001164885	TGCTTTACCTAGAAGATAACAGTGA
				CCAGCTGGCCCACATAATTC
<i>St3gal5</i>	Mus musculus	ST3 beta-galactoside alpha-2,3-sialyltransferase 5	NM_001035228	CAATGGTACACCCGAACCCA
				AGGAGCCAGACTCCAAATGC

<i>Angptl4</i>	Mus musculus	Angiopoietin-like 4	NM_020581	ACTTGGGACCAAGACCATGAC
				TACAGGTACCAAACCACCAGC

Supplemental Table 2. Primers for real-time PCR

Supplemental Table 3

Mice Groups	8-HEPE
HFD	n.d.
HFD with EPA	n.d.
HFD with 8-HEPE	3.7 ± 1.9

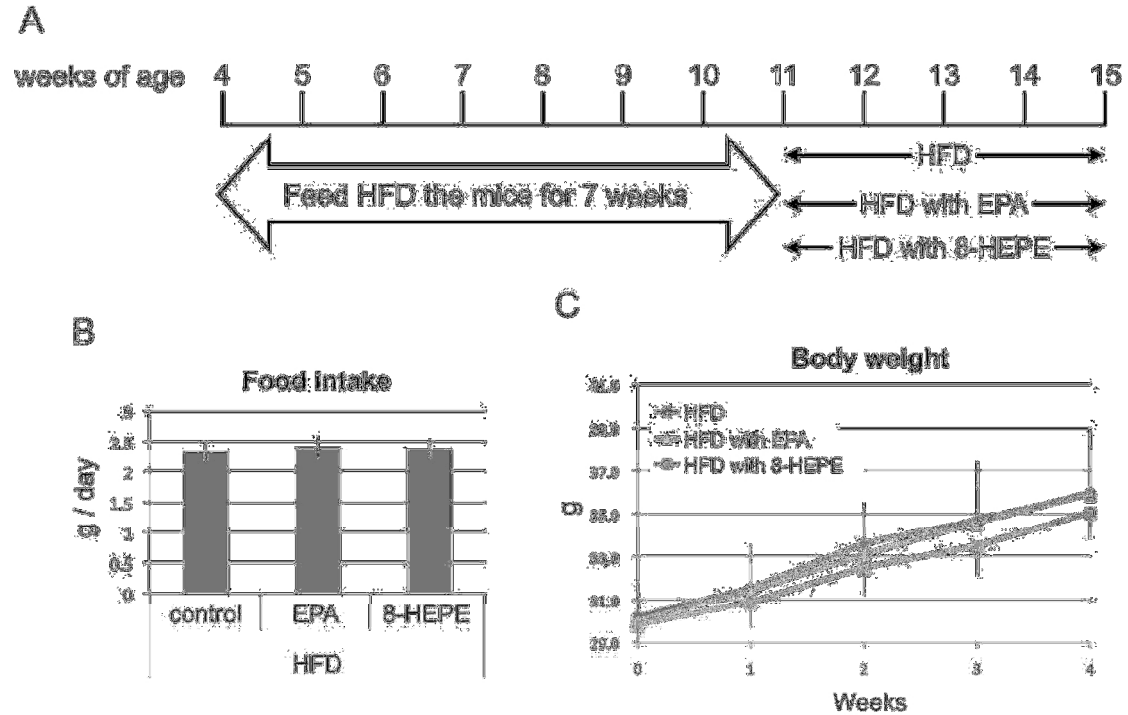
Supplemental Table 3. Concentration (nM) of 8-HEPE in male C57BL/6J-DIO mice fed HFD, HFD with EPA, or HFD with 8-HEPE for 4 wk. Values are means ± SDs, n = 9. Means at a time without a common letter differ, P < 0.05. HFD, High fat diet; HEPE, hydroxyeicosapentaenoic acid; EPA, eicosapentaenoic acid;

Supplemental Table 4

	Plasma adiponectin ( $\mu\text{g} / \text{mL}$ )	Plasma leptin ( $\text{ng} / \text{mL}$ )
HFD	$26.53 \pm 3.09$	$41.25 \pm 10.63$
HFD with EPA	$27.45 \pm 3.61$	$40.17 \pm 14.89$
HFD with 8-HEPE	$26.51 \pm 2.58$	$35.79 \pm 7.07$

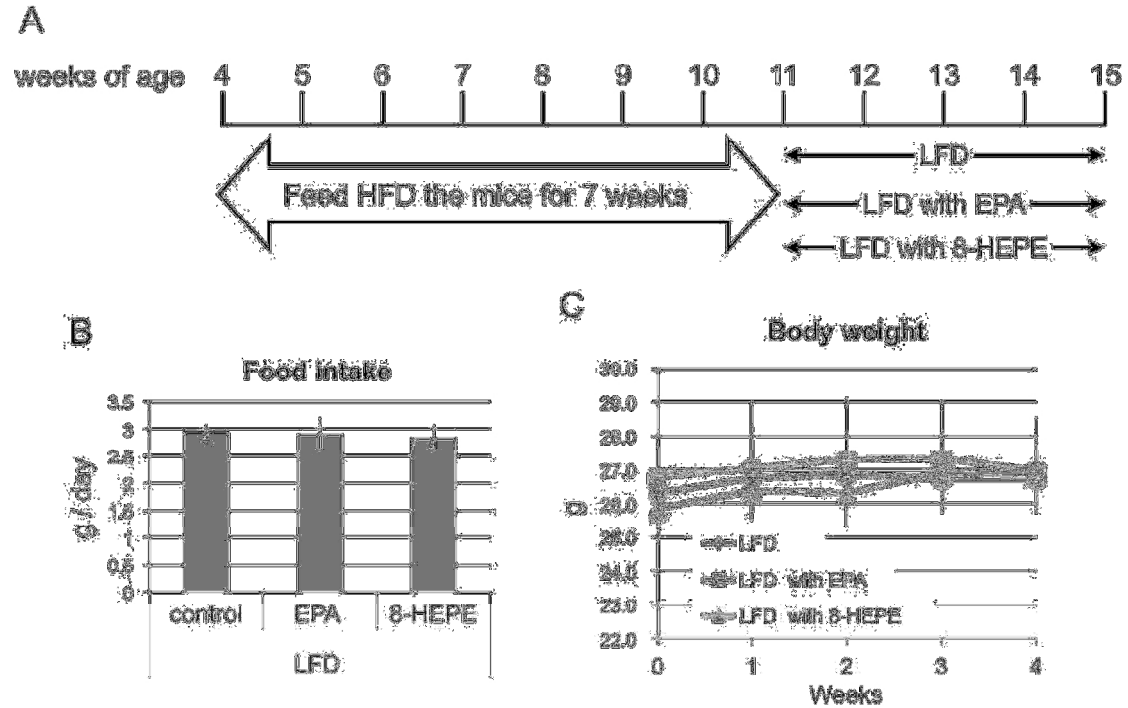
Supplemental Table 4. Concentrations of plasma adiponectin and leptin in male C57BL/6J-DIO mice fed HFD, HFD with EPA or HFD with 8-HEPE for 4 wk. Values are means  $\pm$  SDs, n = 9. HFD, high fat diet; HEPE, hydroxyeicosapentaenoic acid; EPA, eicosapentaenic acid;

Supplemental Fig. 1



Supplemental Figure 1. Experimental design (A), food intake (B) and body weight gain (C) in male C57BL/6J-DIO mice fed HFD, HFD with EPA, or HFD with 8-HEPE for 4wk. Values are means  $\pm$  SDs,  $n = 9$ . HFD, High fat diet; HEPE, hydroxyeicosapentaenoic acid; EPA, eicosapentaenic acid;

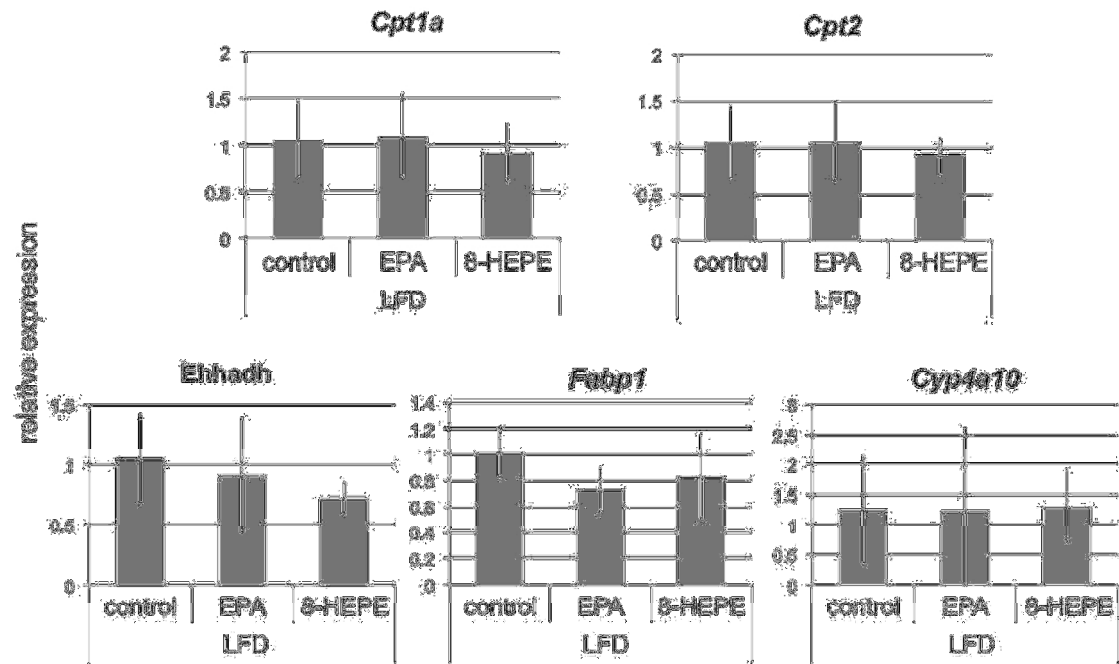
Supplemental Fig. 2



Supplemental Figure 2. Experimental design (A), food intake (B) and body weight gain (C) in male C57BL/6J-DIO mice fed LFD, LFD with EPA, or LFD with 8-HEPE for 4wk. Values are means  $\pm$  SDs, n = 9. LFD, Low fat diet; HEPE, hydroxyeicosapentaenoic acid; EPA, eicosapentaenoic acid;

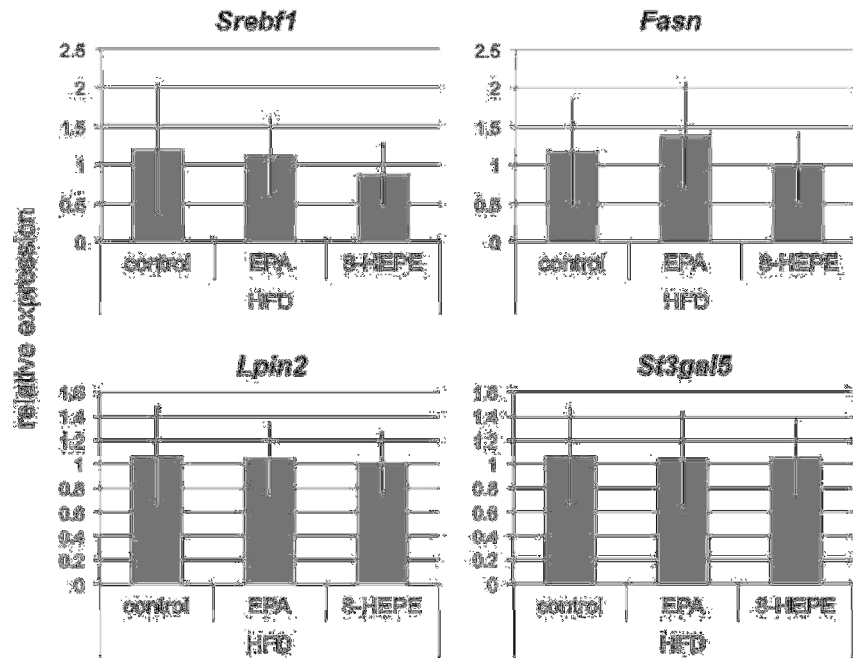


Supplemental Fig. 3



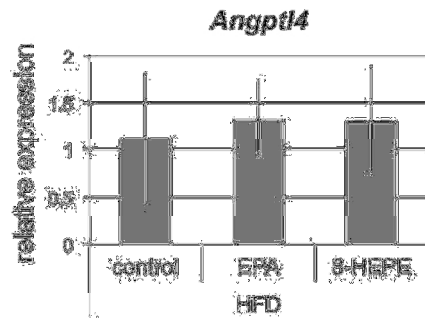
Supplemental Figure 3. Hepatic gene expression changes in male C57BL/6J-DIO mice fed LFD, LFD with EPA, or LFD with 8-HEPE for 4wk. Gene expression levels were measured by real-time PCR and normalized against expression of *Actb*. Values are means  $\pm$  SDs, n = 9. LFD, Low fat diet; HEPE, hydroxyeicosapentaenoic acid; EPA, eicosapentaenoic acid;

Supplemental Fig. 4



Supplemental Figure 4. Hepatic gene expression changes in male C57BL/6J-DIO mice fed HFD, HFD with EPA, or HFD with 8-HEPE for 4wk. Gene expression levels were measured by real-time PCR and normalized against expression of *Actb*. Values are means  $\pm$  SDs, n = 10. HFD, High fat diet; HEPE, hydroxyeicosapentaenoic acid; EPA, eicosapentaenoic acid;

Supplemental Figure 5



Supplemental Figure 5. Muscle *Angptl4* gene expression change in male C57BL/6J-DIO mice fed HFD, HFD with EPA, or HFD with 8-HEPE for 4wk. Gene expression levels were measured by real-time PCR and normalized against expression of *Actb*. Values are means  $\pm$  SDs,  $n = 10$ . HFD, High fat diet; HEPE, hydroxyecosapentaenoic acid; EPA, eicosapentaenic acid;