

ApoC-III ASO Promotes Tissue LPL Activity in Absence of ApoE-Mediated TRL Clearance

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Running Title: ApoE determines the metabolic impact of apoC-III

SUPPLEMENTARY TABLES AND FIGURES.

SUPPLEMENTAL TABLES

Supplemental Table S1: Murine Antisense Oligonucleotide Sequences.

Ionis ID	Name	Target Gene	Sequence
ION 440726	ApoC-III ASO	apoC-III	5'-CCAGCTTTATTAGGGACAGC-3'
ION 141923	Control ASO	N.A. (scramble)	5'-CCTTCCCTGAAGGTCCTCC-3'

Supplemental Table S2: Real-Time PCR Primers.

Gene	Forward Primer	Reverse Primer
<i>Apoc3</i>	5'-TGCAGGGCTACATGGAACAA-3'	5'-TCGGACTCCTGCACGCTACTT-3'
<i>Tbp</i>	5'-GAAGCTGCGGTACAATTCCAG- 3'	5'-CCCCTTGTACCCTTCACCAAT-3'

Supplemental Table S3: Lipid analysis of a homozygote apoE cohort administered with placebo or volanesorsen (Ionis 304801) as a monotherapy.

ApoE isoform	Treatment		Parameter				
			ApoC-III (mg/dL)	TGs (mg/dL)	HDL-C (mg/dL)	non-HDL-C (mg/dL)	
E2/E2	Placebo (n = 1)	Pre	11.0	190.0	41.0	101.0	
		Post	13.8	244.0	39.0	124.0	
		Change	26.0%	28.4%	-4.9%	22.8%	
	Volanesorsen 300 mg (n = 2)	Pre	22.6 ± 4.1	687 ± 374.8	40 ± 17.0	245 ± 94.8	
		Post	3.4 ± 0.9	118.5 ± 1.4	68.8 ± 29.3	78.5 ± 14.9	
		Change	-85.3 ± 1.5%	-79.8 ± 10.8%	71.8 ± 0.5%	-66.6 ± 6.8%	
	E3/E3	Placebo (n = 6)	Pre	20.2 ± 6.9	408.8 ± 153.8	33.5 ± 6.2	219.5 ± 58.7
			Post	18.8 ± 5.6	396.7 ± 148	36.4 ± 9.5	201.7 ± 20.3
			Change	-2.3 ± 29.0%	1.1 ± 27.6%	8.3 ± 18.7%	-5.1 ± 15.8%
Volanesorsen 100 mg (n = 3)		Pre	27.6 ± 0.9	936.2 ± 109.8	26.0 ± 2.0	258.0 ± 19.7	
		Post	9.9 ± 4.7	249.5 ± 118.5	41.3 ± 15.5	211.8 ± 14.7	
		Change	-64.6 ± 16.2%	-72.8 ± 14.3%	60.0 ± 61.1%	-17.6 ± 8.2%	
Volanesorsen 200 mg (n = 3)		Pre	23.0 ± 2.2	599.2 ± 224.2	30.7 ± 5.8	170.7 ± 62.7	
		Post	7.4 ± 0.4	202.2 ± 60.8	41.8 ± 6.8	158.2 ± 28.1	
		Change	-67.4 ± 4.6%	-63.0 ± 18.5%	37.9 ± 19.1%	-3.5 ± 15.7%	
E4/E4	Placebo (n = 0)	Pre	-	-	-	-	
		Post	-	-	-	-	
		Change	-	-	-	-	
	Volanesorsen 300 mg (n = 3)	Pre	20.4 ± 2.6	462.5 ± 129.5	30.7 ± 1.2	195.7 ± 11.0	
		Post	4.3 ± 1.6	113 ± 7.7	45 ± 1.7	178.8 ± 36.8	
		Change	-78.1 ± 10.3%	-74.1 ± 7.8%	47 ± 10.9%	-8.7 ± 17.7%	

Values represent mean ± SD.

Supplemental Table S4: Lipid analysis of a homozygote apoE cohort administered with placebo or volanesorsen (Ionis 304801) as an add-on to fibrates.

ApoE isoform	Treatment		Parameter			
			ApoC-III (mg/dL)	TGs (mg/dL)	HDL-C (mg/dL)	non-HDL-C (mg/dL)
E2/E2	Fibrate + Placebo (n = 3)	Pre	18.6 ± 5.4	482.7 ± 222.9	36.7 ± 13.3	173.7 ± 65.5
		Post	18.5 ± 5.5	412.2 ± 211.8	42.3 ± 11.9	159.2 ± 50.0
		Change	0.8 ± 19.0%	-10.6 ± 28.9%	19.0 ± 29.8%	-5.4 ± 15.4%
	Fibrate + Volanesorsen 300 mg (n = 1)	Pre	11.6	241.0	47.0	178.0
		Post	3.0	91.0	63.5	75.0
		Change	-74.3%	-62.2%	35.1%	-57.9%
E3/E3	Fibrate + Placebo (n = 1)	Pre	10.3	204.0	37.0	178.0
		Post	15.3	314.0	30.0	179.0
		Change	48.4%	53.9%	-18.9%	0.6%
	Fibrate + Volanesorsen 200 mg (n = 4)	Pre	13.5 ± 1.0	262.1 ± 40.0	34.8 ± 3.8	188.0 ± 33.8
		Post	4.9 ± 1.9	126.5 ± 30.9	50.5 ± 10.1	145.4 ± 22.3
		Change	-63.8 ± 13.6%	-51.3 ± 11.3%	44.8 ± 21.0%	-21.8 ± 10.4%
	Fibrate + Volanesorsen 300 mg (n = 3)	Pre	15.7 ± 1.8	255.2 ± 17.1	39.7 ± 14.6	165.3 ± 47.0
		Post	5.5 ± 2.3	98.0 ± 30.4	61.2 ± 29.3	134.5 ± 19.0
		Change	-64.0 ± 16.5%	-61.0 ± 14.0%	51.1 ± 44.9%	-15.5 ± 18.0%
E4/E4	Fibrate + Placebo (n = 1)	Pre	25.5	495.0	30.0	316.0
		Post	21.4	408.0	31.0	281.0
		Change	-16.2%	-17.6%	3.3%	-11.1%
	Fibrate + Volanesorsen 300 mg (n = 1)	Pre	29.6	932.0	14.0	235.0
		Post	5.9	249.5	21.5	183.0
		Change	-80.1%	-73.2%	53.6%	-22.1%

Values represent mean ± SD.

Supplemental Table S5: Combined data of lipid analysis of a heterozygote apoE cohort administered with placebo or volanesorsen (Ionis 304801) as a monotherapy or add on to fibrates.

ApoE isoform	Treatment		Parameter			
			ApoC-III (mg/dL)	TGs (mg/dL)	HDL-C (mg/dL)	non-HDL-C (mg/dL)
E2/E3	Placebo (n = 5)	Pre	25.2 ± 10.7	760.2 ± 624.4	30.4 ± 10.9	241.2 ± 112.3
		Post	16.7 ± 4.4	489.0 ± 361.5	30.9 ± 10.5	182.6 ± 46.0
		Change	-23.4 ± 39.1%	-10.5 ± 78.5%	2.3 ± 10.7%	-15.2 ± 36.2%
	Volanesorsen 100 mg (n = 2)	Pre	12.0 ± 3.6	420.0 ± 174.0	37.0 ± 1.4	130.5 ± 55.9
		Post	9.4 ± 3.1	390.8 ± 161.6	35.8 ± 10.3	125.0 ± 20.5
		Change	-21.6 ± 2.0%	-7.0 ± 0.1%	-3.8 ± 24.0%	1.7 ± 27.8%
	Volanesorsen 200 mg (n = 2)	Pre	21.3 ± 12.0	594.0 ± 418.6	31.0 ± 4.2	199.5 ± 26.2
		Post	5.8 ± 0.1	166.5 ± 2.1	43.0 ± 0.7	200.5 ± 33.9
		Change	-67.5 ± 17.9%	-62.9 ± 25.8%	39.9 ± 16.9%	2.5 ± 30.5%
	Volanesorsen 300 mg (n = 3)	Pre	25.0 ± 5.8	491.3 ± 71.2	36.0 ± 2.0	204.7 ± 33.7
		Post	6.5 ± 2.9	164.8 ± 31.2	55.5 ± 3.5	123.3 ± 55.4
		Change	-74.8 ± 7.3%	-65.7 ± 9.9%	54.3 ± 9.0%	-40.9 ± 19.7%
E2/E4	Placebo (n = 0)	Pre	-	-	-	-
		Post	-	-	-	-
		Change	-	-	-	-
	Volanesorsen 200 mg (n = 2)	Pre	24.0 ± 2.8	715.8 ± 1.8	35.0 ± 4.2	178.0 ± 2.8
		Post	10.5 ± 10.3	424.5 ± 419.3	37.8 ± 12.4	176.5 ± 13.4
		Change	-53.3 ± 48.6%	-40.6 ± 58.7%	10.8 ± 48.8%	-0.8 ± 9.1%
E3/E4	Placebo (n = 7)	Pre	22.4 ± 2.8	489.6 ± 83.6	33.7 ± 5.4	176.7 ± 50.0
		Post	27.0 ± 13.7	671.3 ± 417.9	31.6 ± 5.1	188.6 ± 48.5
		Change	17.2 ± 44.1%	39.0 ± 88.4%	-5.7 ± 11.5%	15.1 ± 53.3%
	Volanesorsen 100 mg (n = 6)	Pre	23.2 ± 7.3	474.8 ± 158.8	31.7 ± 2.0	201.3 ± 30.3
		Post	14.0 ± 5.8	316.3 ± 158.0	38.1 ± 5.7	193.0 ± 52.4
		Change	-33.9 ± 37.3%	-18.6 ± 70.1%	20.0 ± 13.7%	-4.9 ± 20.4%
	Volanesorsen 200 mg (n = 8)	Pre	20.8 ± 6.4	533.4 ± 375.4	32.1 ± 5.4	194.6 ± 72.5
		Post	7.4 ± 3.5	188.6 ± 83.3	49.1 ± 18.8	163.1 ± 47.1
		Change	-61.0 ± 20.2%	-56.1 ± 24.3%	49.3 ± 32.4%	-11.7 ± 21.1%
	Volanesorsen	Pre	21.3 ± 7.4	520.7 ± 249.6	30.6 ± 10.8	147.6 ± 51.2

300 mg (n = 7)	Post Change	4.6 ± 2.0 -75.4 ± 14.6%	155.8 ± 46.3 -64.5 ± 15.5%	41.3 ± 9.3 42.2 ± 28.0%	168.2 ± 75.9 11.6 ± 29.3%
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Values represent mean ± SD.

Supplemental Table S6: Lipid analysis of a heterozygote apoE cohort administered with placebo or volanesorsen (Ionis 304801) as a monotherapy.

ApoE isoform	Treatment		Parameter			
			ApoC-III (mg/dL)	TGs (mg/dL)	HDL-C (mg/dL)	non-HDL-C (mg/dL)
E2/E3	Placebo (n = 3)	Pre	29.6 ± 12.2	915.7 ± 786.0	28.7 ± 13.3	268.7 ± 147.7
		Post	17.8 ± 5.8	603.5 ± 458.4	28.2 ± 13.3	211.5 ± 12.1
		Change	-27.0 ± 53.7%	-1.0 ± 105.5%	-1.7 ± 7.1%	-4.6 ± 46.3%
	Volanesorsen 100 mg (n = 2)	Pre	12.0 ± 3.6	420.0 ± 174.0	37.0 ± 1.4	130.5 ± 55.9
		Post	9.4 ± 3.1	390.8 ± 161.6	35.8 ± 10.3	125.0 ± 20.5
		Change	-21.6 ± 2.0%	-7.0 ± 0.1%	-3.8 ± 24.0%	1.7 ± 27.8%
	Volanesorsen 200 mg (n = 2)	Pre	21.3 ± 12.0	594.0 ± 418.6	31.0 ± 4.2	199.5 ± 26.2
		Post	5.8 ± 0.1	166.5 ± 2.1	43.0 ± 0.7	200.5 ± 33.9
		Change	-67.2 ± 17.9%	-62.9 ± 25.8%	39.9 ± 16.9%	2.5 ± 30.5%
	Volanesorsen 300 mg (n = 1)	Pre	30.3	573.0	38.0	180.0
		Post	8.6	151.5	55.5	118.5
		Change	-71.6%	-73.6%	46.1%	-34.2%
E2/E4	Placebo (n = 0)	Pre	-	-	-	-
		Post	-	-	-	-
		Change	-	-	-	-
	Volanesorsen 200 mg (n = 2)	Pre	24.0 ± 2.8	715.8 ± 1.8	35.0 ± 4.2	178.0 ± 2.8
		Post	10.5 ± 10.3	424.5 ± 419.3	37.8 ± 12.4	176.5 ± 13.4
		Change	-53.3 ± 48.6%	-40.6 ± 58.7%	10.8 ± 48.8%	-0.8 ± 9.1%
E3/E4	Placebo (n = 6)	Pre	22.2 ± 3.0	495.4 ± 90.0	33.3 ± 5.8	184.3 ± 50.1
		Post	28.3 ± 14.5	719.8 ± 435.7	31.5 ± 5.6	201.6 ± 37.4
		Change	22.7 ± 45.5%	48.2 ± 93.0%	-4.8 ± 12.3%	20.2 ± 56.5%
	Volanesorsen 100 mg (n = 6)	Pre	23.2 ± 7.3	474.8 ± 158.8	31.7 ± 2.0	201.3 ± 30.3
		Post	14.0 ± 5.8	316.3 ± 158.0	38.1 ± 5.7	193.0 ± 52.4
		Change	-33.9 ± 37.3%	-18.6 ± 70.1%	20.0 ± 13.7%	-4.9 ± 20.5%
	Volanesorsen 200 mg (n = 5)	Pre	23.1 ± 6.4	667.0 ± 423.8	31.6 ± 3.0	227.8 ± 69.3
		Post	7.3 ± 3.8	205.5 ± 71.3	45.6 ± 10.2	186.9 ± 42.0
		Change	-64.4 ± 24.7%	-59.3 ± 28.6%	43.9 ± 24.6%	-14.9 ± 16.3%
	Volanesorsen 300 mg	Pre	22.4 ± 8.5	563.2 ± 259.6	32.0 ± 11.1	134.2 ± 44.8
		Post	4.0 ± 1.8	161.3 ± 43.7	40.6 ± 7.0	154.7 ± 62.0

(n = 5) **Change** $-79.9 \pm 11.4\%$ $-65.0 \pm 18.6\%$ $34.3 \pm 29.7\%$ $13.9 \pm 32.2\%$

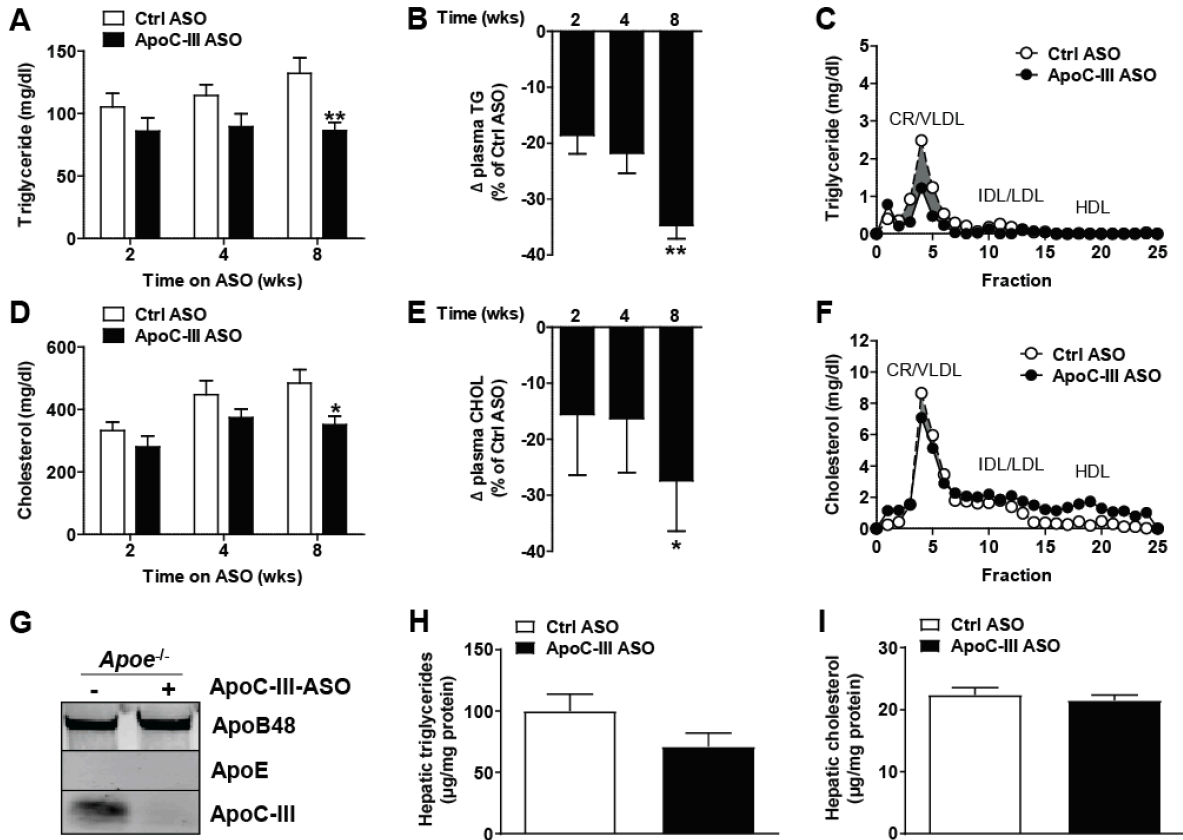
Values represent mean \pm SD.

Supplemental Table S7: Lipid analysis of a heterozygote apoE cohort administered with placebo or volanesorsen (Ionis 304801) as add on to fibrates.

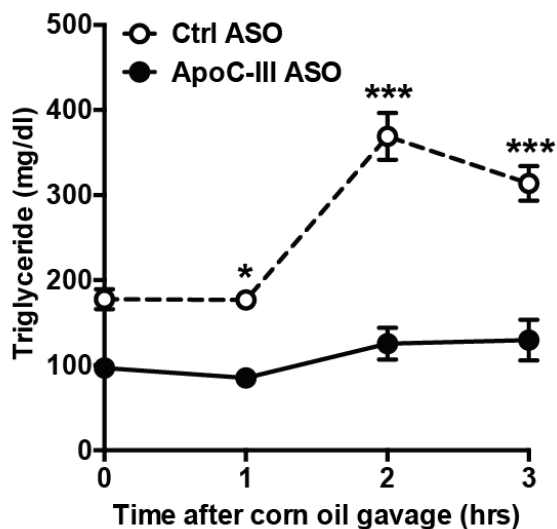
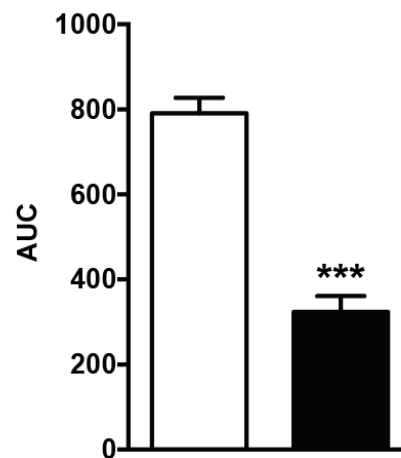
ApoE isoform	Treatment		Parameter			
			ApoC-III (mg/dL)	TGs (mg/dL)	HDL-C (mg/dL)	non-HDL-C (mg/dL)
E2/E3	Fibrate + Placebo (n = 2)	Pre	18.6 ± 3.4	527.0 ± 377.6	33.0 ± 9.9	200.0 ± 33.9
		Post	15.0 ± 0.1	317.3 ± 64.0	35.0 ± 5.7	139.3 ± 43.5
		Change	-17.8 ± 15.7%	-24.9 ± 41.7%	8.4 ± 15.4%	-31.2 ± 10.1%
	Fibrate + Volanesorsen 300 mg (n = 2)	Pre	22.3 ± 5.0	450.5 ± 12.0	35.0 ± 1.4	217.0 ± 36.8
		Post	5.5 ± 3.3	171.5 ± 41.0	55.5 ± 5.0	125.8 ± 78.1
		Change	-76.5 ± 9.5%	-61.8 ± 10.1%	58.4 ± 7.7%	-44.3 ± 26.6%
E2/E4	Fibrate + Placebo (n = 0)	Pre	-	-	-	-
		Post	-	-	-	-
		Change	-	-	-	-
	Fibrate + Volanesorsen (n = 0)	Pre	-	-	-	-
		Post	-	-	-	-
		Change	-	-	-	-
E3/E4	Fibrate + Placebo (n = 1)	Pre	23.4	455.0	36.0	131.0
		Post	19.6	380.5	32.0	110.5
		Change	-16.2%	-16.4%	-11.1%	-15.6%
	Fibrate + Volanesorsen 200 mg (n = 3)	Pre	16.9 ± 5.2	310.7 ± 122.3	33.0 ± 9.0	139.3 ± 37.8
		Post	7.7 ± 3.7	160.5 ± 110.5	54.8 ± 30.8	123.3 ± 22.5
		Change	-55.3 ± 11.5%	-50.7 ± 18.8%	58.3 ± 47.6%	-6.4 ± 31.0%
	Fibrate + Volanesorsen 300 mg (n = 2)	Pre	18.5 ± 4.5	414.5 ± 269.4	27.0 ± 12.7	181.0 ± 67.9
		Post	6.2 ± 2.1	142.0 ± 68.6	43.0 ± 17.7	202.0 ± 126.6
		Change	-64.2 ± 20.0%	-63.4 ± 7.3%	61.8 ± 10.8%	5.9 ± 30.2%

Values represent mean ± SD.

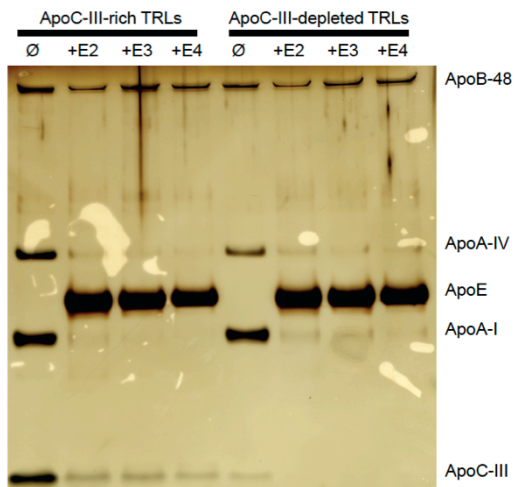
Supplemental Figures:



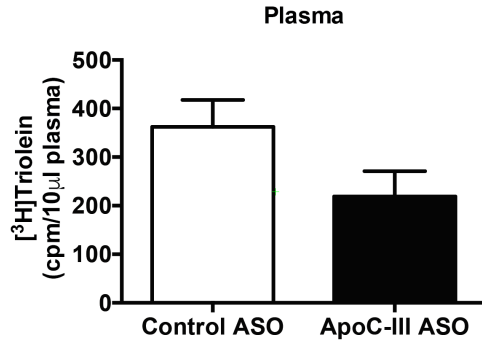
Supplemental Figure S1. Targeting *apoC-III* with ASOs in chow-fed *Apoe*^{-/-} mice. (A) *Apoe*^{-/-} mice were administered once weekly with apoC-III ASO or control ASO (50 mg/kg bodyweight) for a period of 8 weeks. Fasting plasma TG levels were measured at the indicated time points. (B) ApoC-III ASO mediated relative change of plasma TG levels compared with control ASO. (C) Pooled plasma samples after 4 weeks of ASO treatment were analyzed by size-exclusion FPLC and TGs in each fraction were measured. (D) Fasting plasma cholesterol levels after apoC-III ASO treatment and (E) relative change in cholesterol compared to control ASO. (F) FPLC analysis of pooled plasma samples 4 weeks of ASO treatment followed by cholesterol determination in each fraction. (G) VLDLs were isolated by ultracentrifugation and pooled VLDL samples (5 μg protein/lane) were analyzed by Western blotting with antibodies against apoB, apoC-III and apoE. Hepatic TG (H) and cholesterol (I) levels were measured (n = 8-10/group, values represent mean ± SEM, *p<0.05, **p<0.01).

A**B**

Supplemental Figure S2. Fat Tolerance Test in Western Diet Fed *ApoE*^{-/-}*Ndst1*^{fl/fl}*Alb-Cre*⁺ mice. (A-B) ApoC-III ASO or control ASO (50 mg/kg bodyweight) were administered to *ApoE*^{-/-}*Ndst1*^{fl/fl}*Alb-Cre*⁺ mice fed a Western-type diet for 8 weeks and *ApoE*^{-/-}*Ndst1*^{fl/fl}*Alb-Cre*⁺ mice. Fasted mice (5h) were given a 250 μ L bolus of corn oil by oral gavage to measure fat tolerance. Postprandial TG levels were measured 0, 1, 2, and 3 hours after gavage (n = 4/group).



Supplemental Figure S3: ApoE isoform reconstitution of murine apoE-deficient TRLs. Silver stained gel of [³H]TRLs reconstituted with either apoE2, E3 or E4 (apoE to [³H]TRL ratio 1:2).



Supplemental Figure S4. ApoC-III lowering decreased [³H]triolein labeled liposyn particles in *ApoE*^{-/-}*Ndst1*^{ff}*Alb-Cre*⁺ mice. *ApoE*^{-/-}*Ndst1*^{ff}*Alb-Cre*⁺ mice were fasted for 5 h. After a 5 min intravenous injection of [³H]triolein labeled liposyn particles (100 µl) blood was collected. [³H]triolein plasma concentrations were determined by liquid scintillation counting (n = 4-6).