

Apolipoprotein Mimetic Peptides

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National Institutes of Health

(No relevant disclosures)



National Institutes
of Health



National Heart, Lung,
and Blood Institute

Overview

- Apolipoprotein Mimetic Peptides
- ApoC-II Mimetic Peptide
- ApoC-II KO Mice and peptide treatment

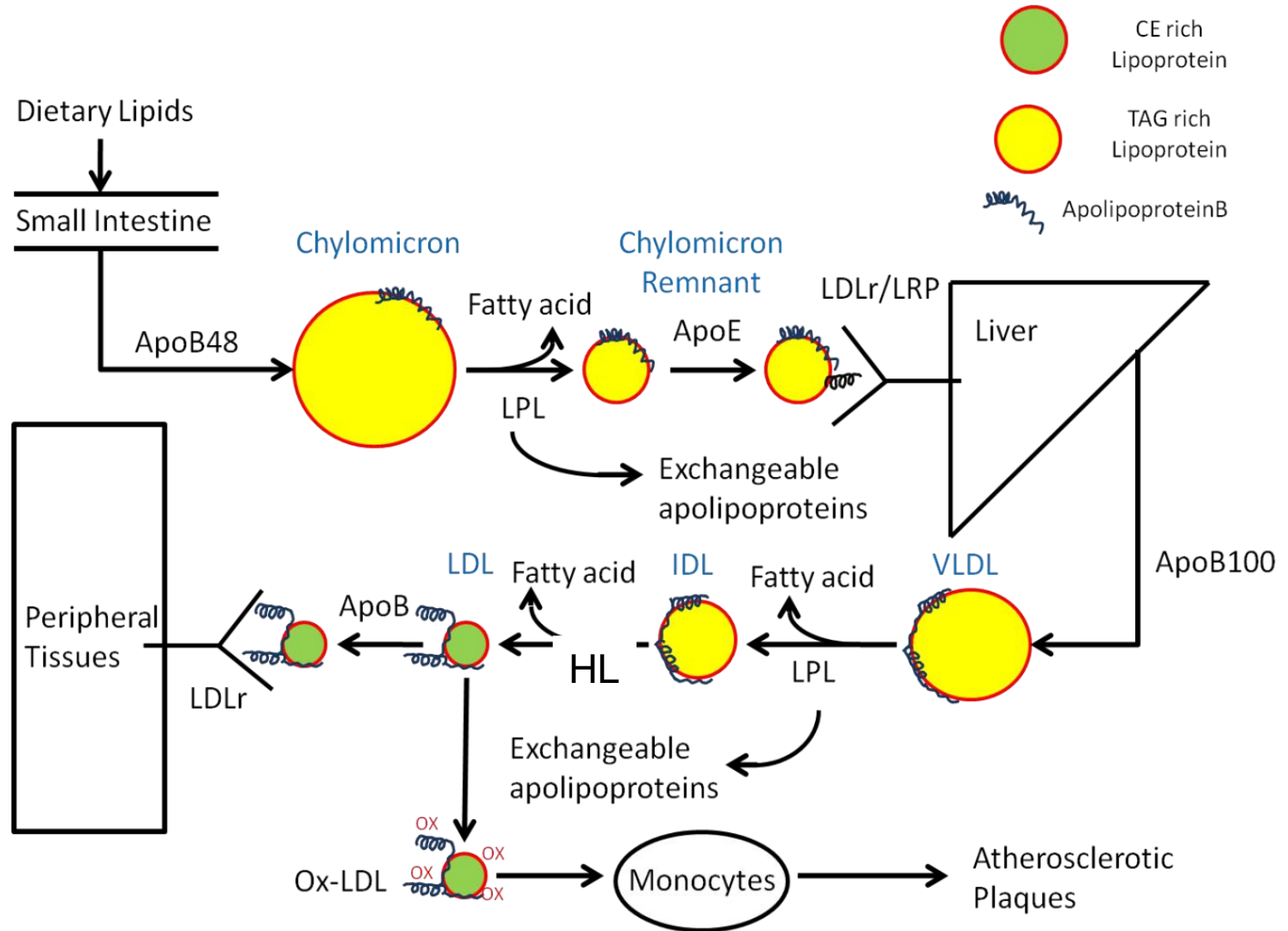
Apolipoprotein Mimetic Peptides

- Sequestration oxidized lipids: D4F, L4F
- Cholesterol efflux: 5A, ATI-5261
- LCAT activation: ESP24218
- ApoB particle uptake: E-18A

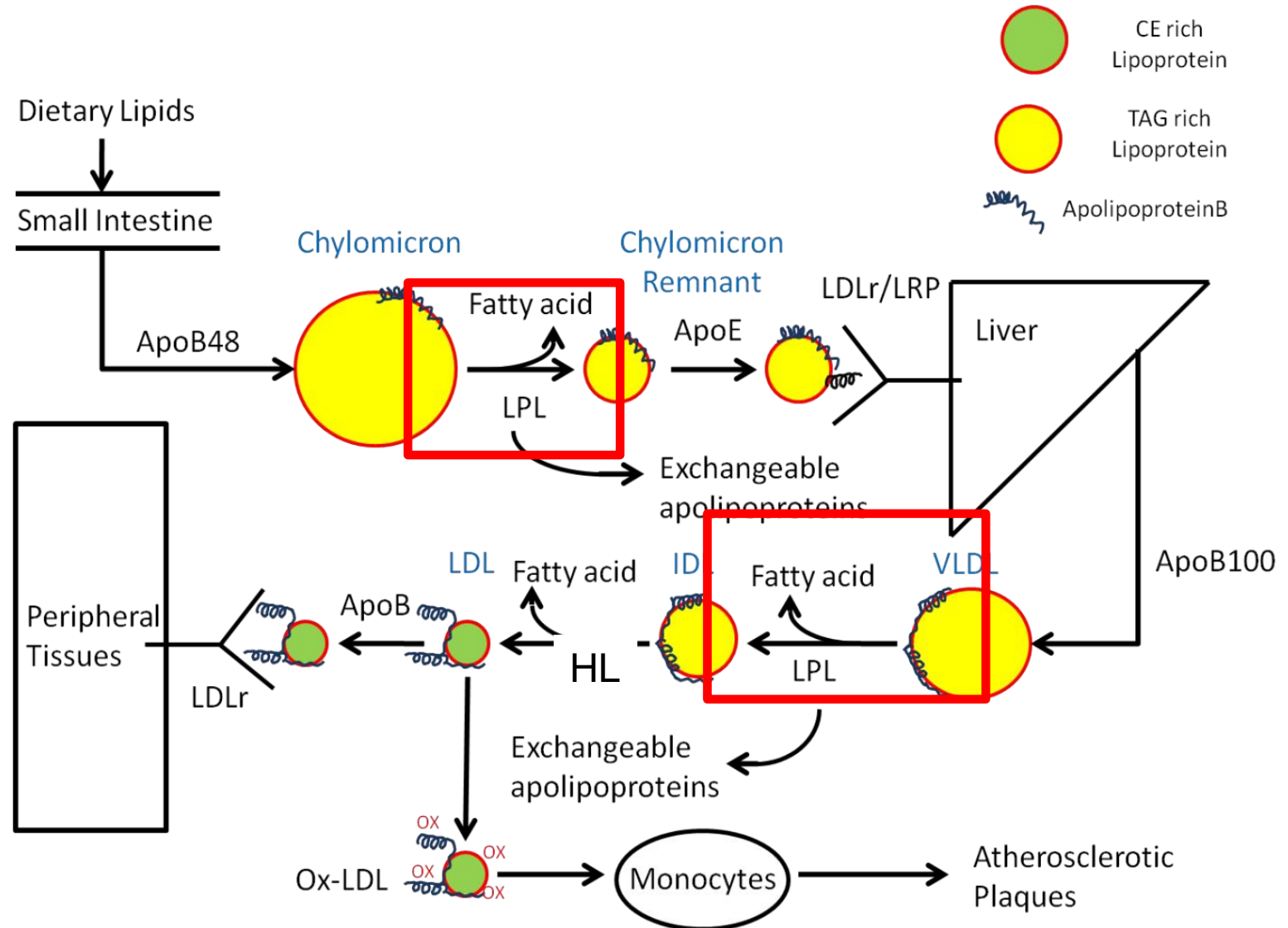
Overview

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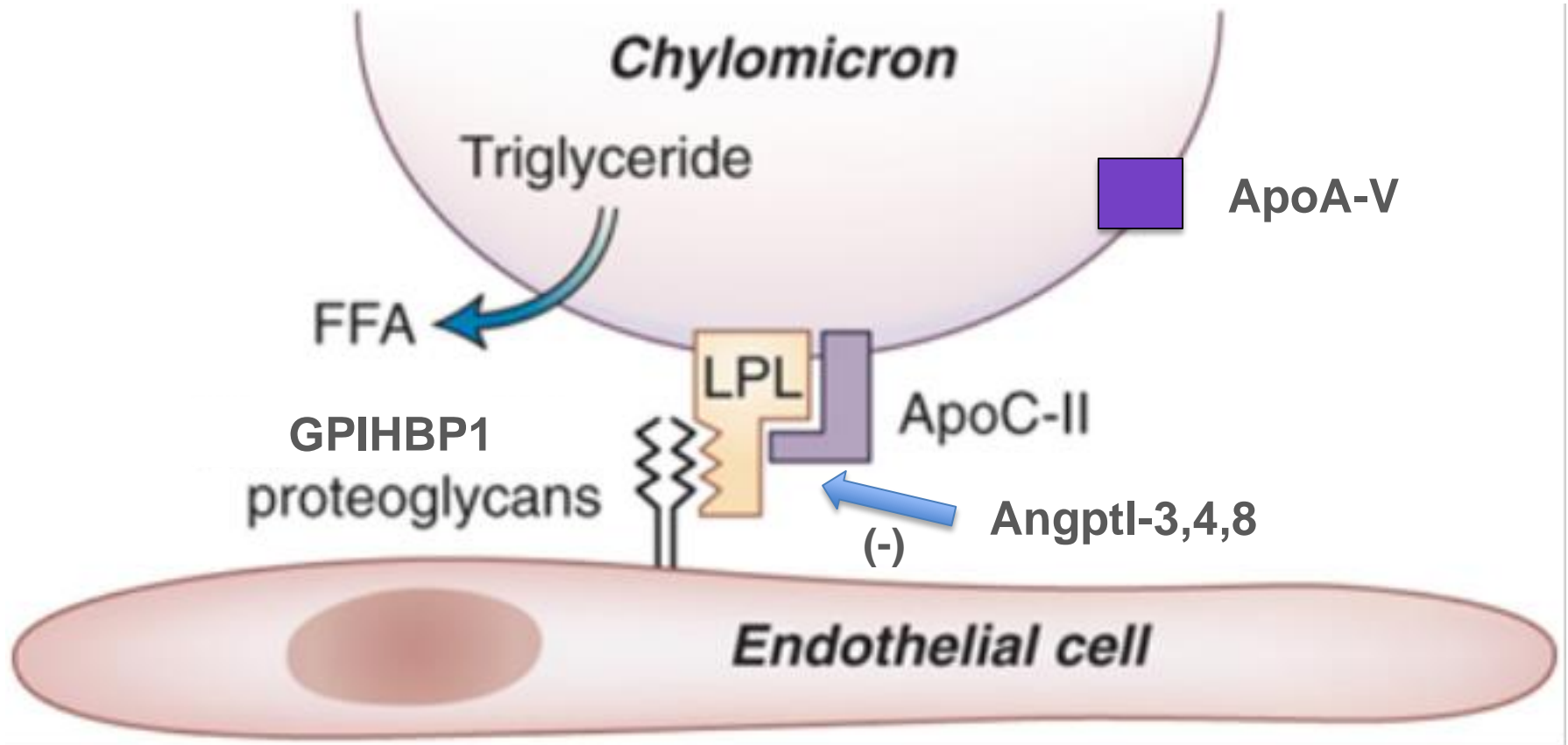
Catabolism of TG-Rich Lipoproteins



Catabolism of TG-Rich Lipoproteins



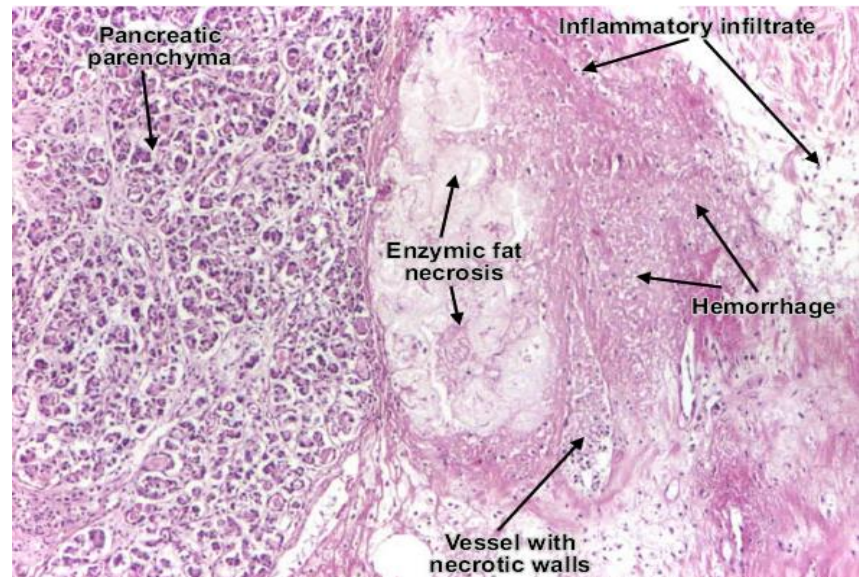
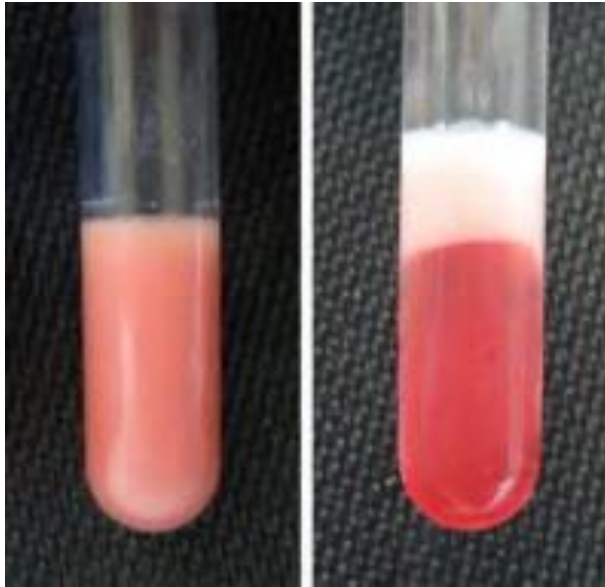
LPL-CII Lipolytic Complex



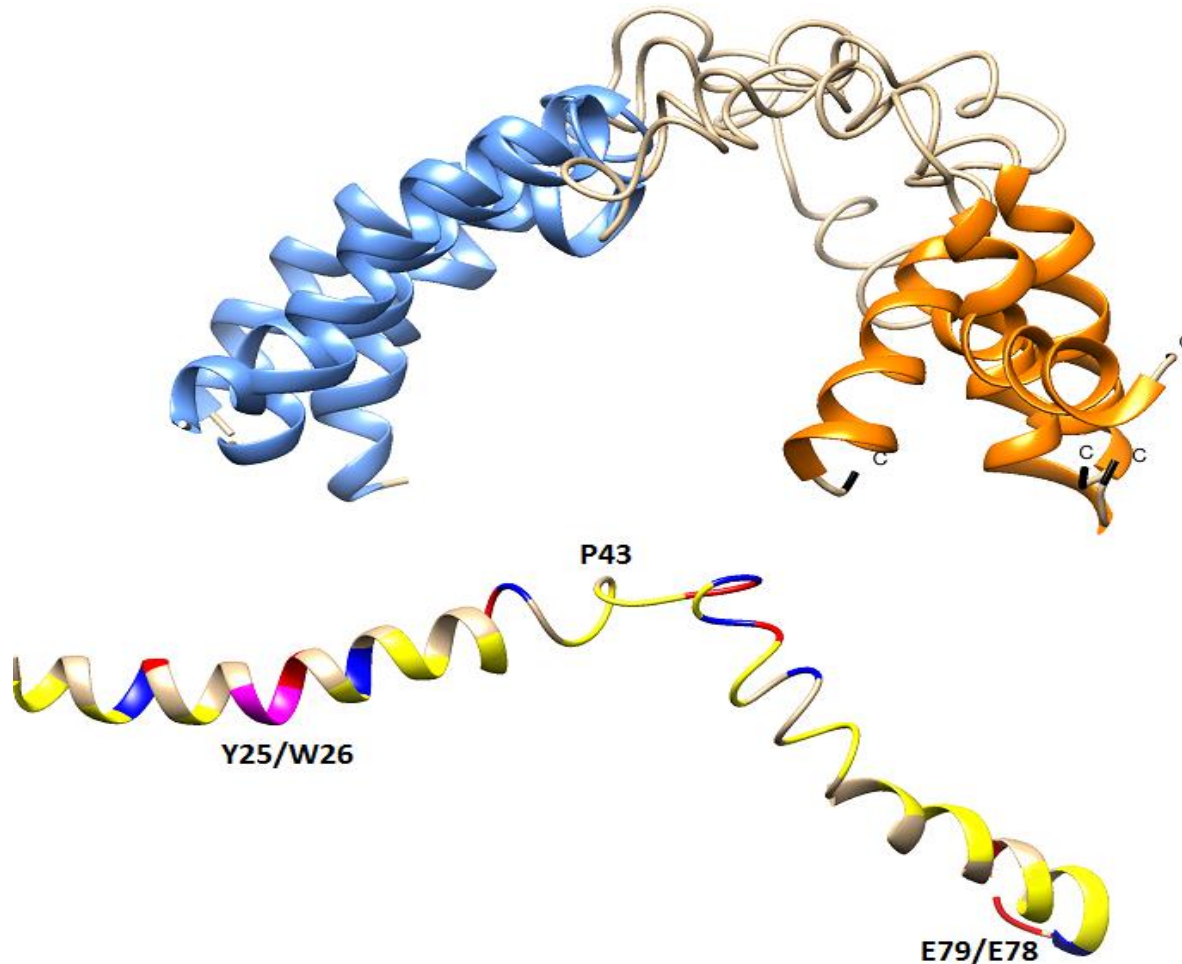
Genetic Causes of Hypertriglyceridemia

	Inheritance/ Population Freq	Pathogenesis	Typical Lipid/ Lipoprotein Particles	Comments
LPL deficiency (Familial Type I)	Auto Recessive (1 in 1,000,000)	Increased CM due to (-) LPL or LPL inhibitor	Homozygotes TG/Chol 10:1 TG > 1,000	Lipemia retinalis, HSM, eruptive xanthomas
Apo CII deficiency	Auto Recessive (rare)	Absence of LPL cofactor, apo CII	Homozygotes TG/Chol 10:1 TG > 1,000	HSM & xanthomas less common than LPL def
Apo AV homozygosity	rare	Mutations->AV truncation w/o lipid-binding domains at carboxy terminus	Homozygotes TG/Chol 10:1 TG > 1,000	Can be late onset incomplete penetrance and resistance to tx
GPIHBP1	Rare, expressed in childhood	Reduced binding to LPL	TG/Chol 7:1 TG>500	Partially responsive to low fat diet

Clinical Features of Hypetriglyceridemia



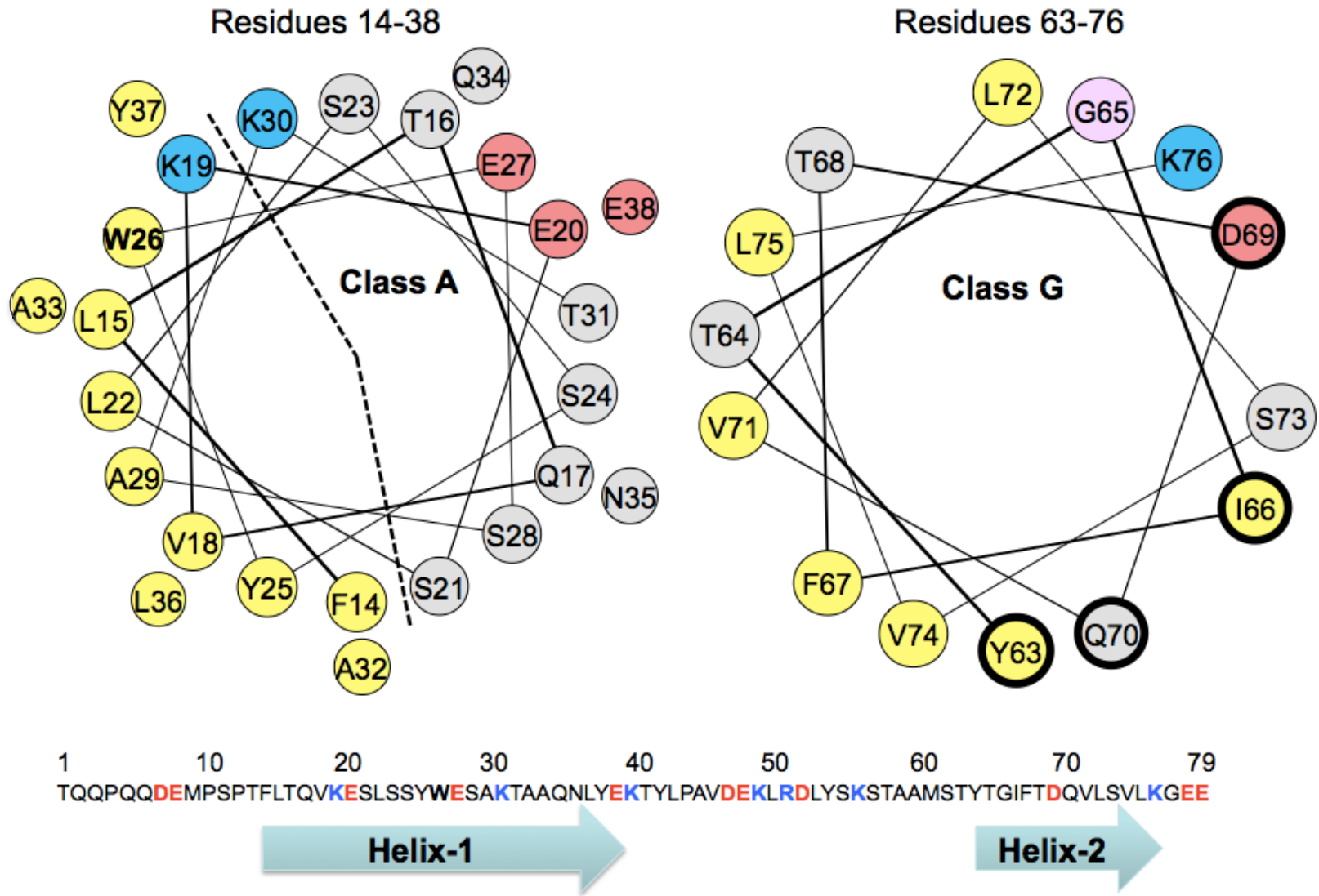
ApoC-II Structure



- 79 amino acids
- 60% helical content is acquired for binding to lipid

MacRaid, C., et al. 2001. *Biochemistry* **40**: 5414-21.

ApoC-II Structure

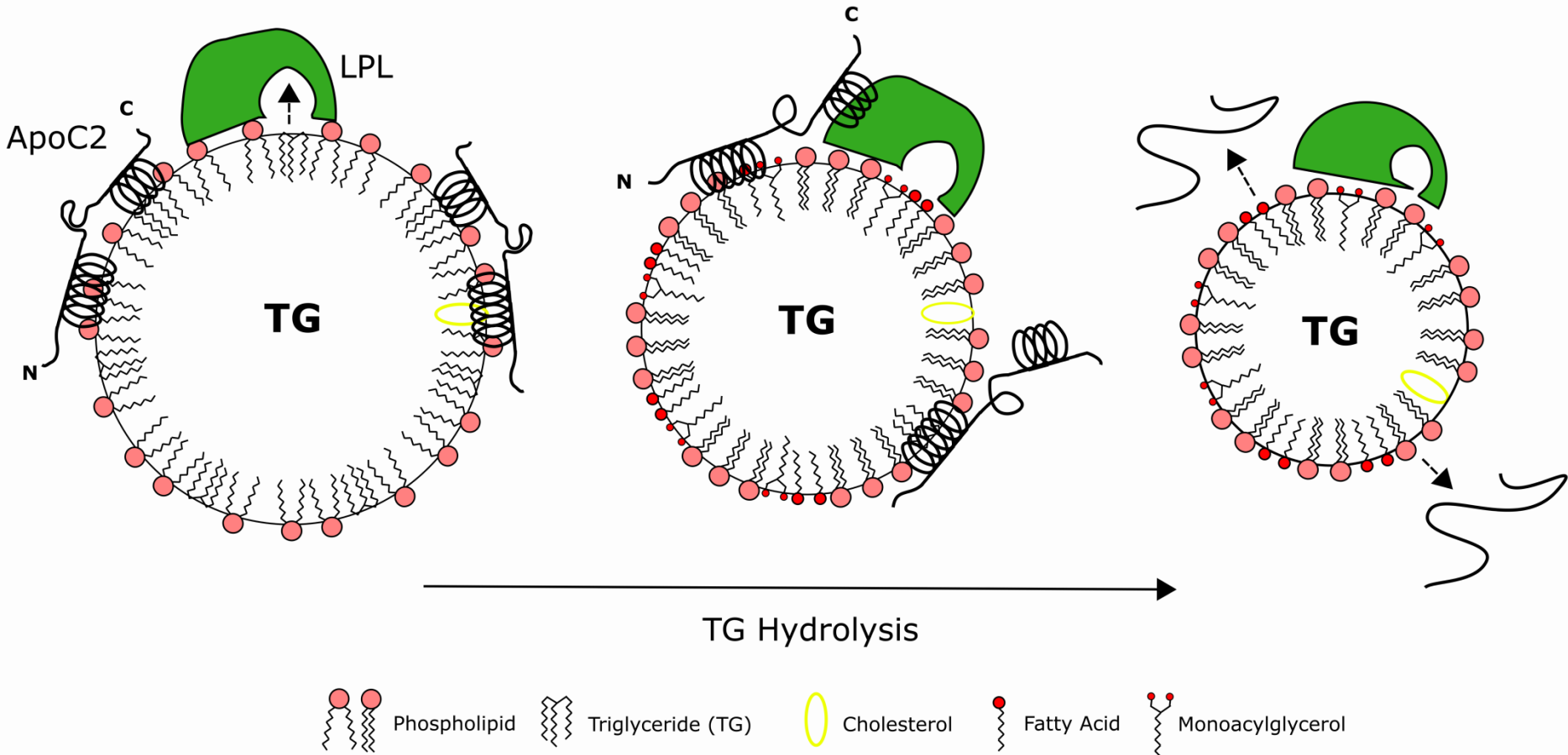


Model for LPL Mechanism of Activation

ApoC2 is not Required for LPL Interaction with Lipid

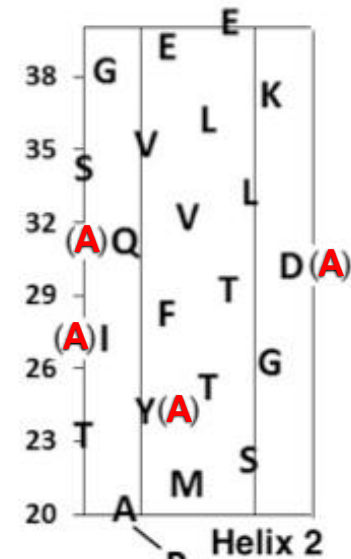
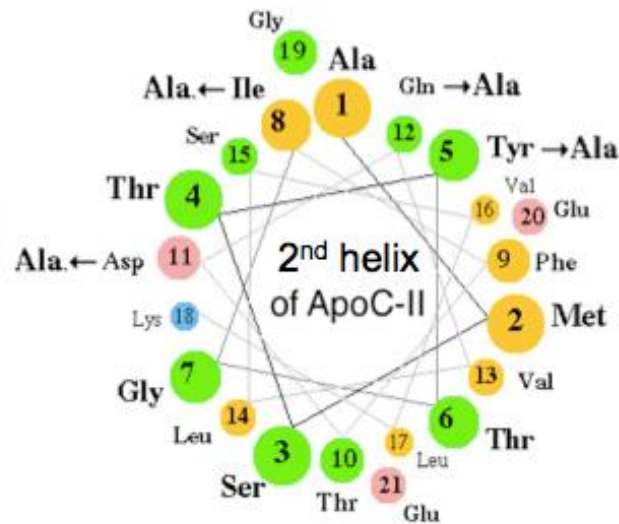
ApoC2 Induces Active LPL Structure during Hydrolysis

ApoC2 Dissociates and LPL is Inactive

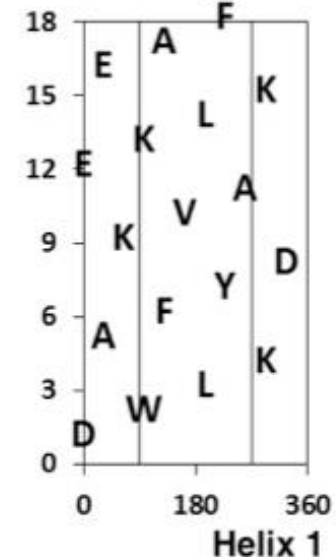
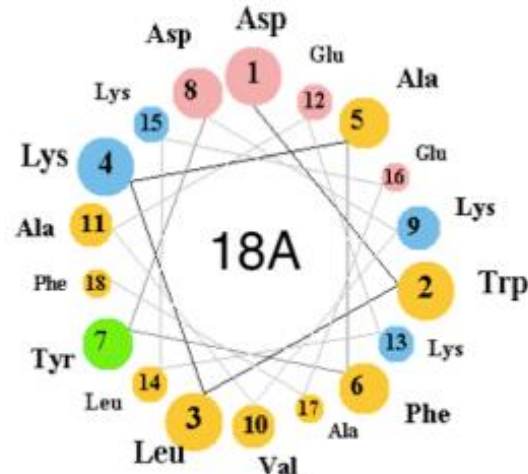


A Bi-helical ApoC-II Mimetic Peptide (CII-a)

LPL activating motif



Lipoprotein anchoring motif



Predicted Structure and Interaction with Lipid

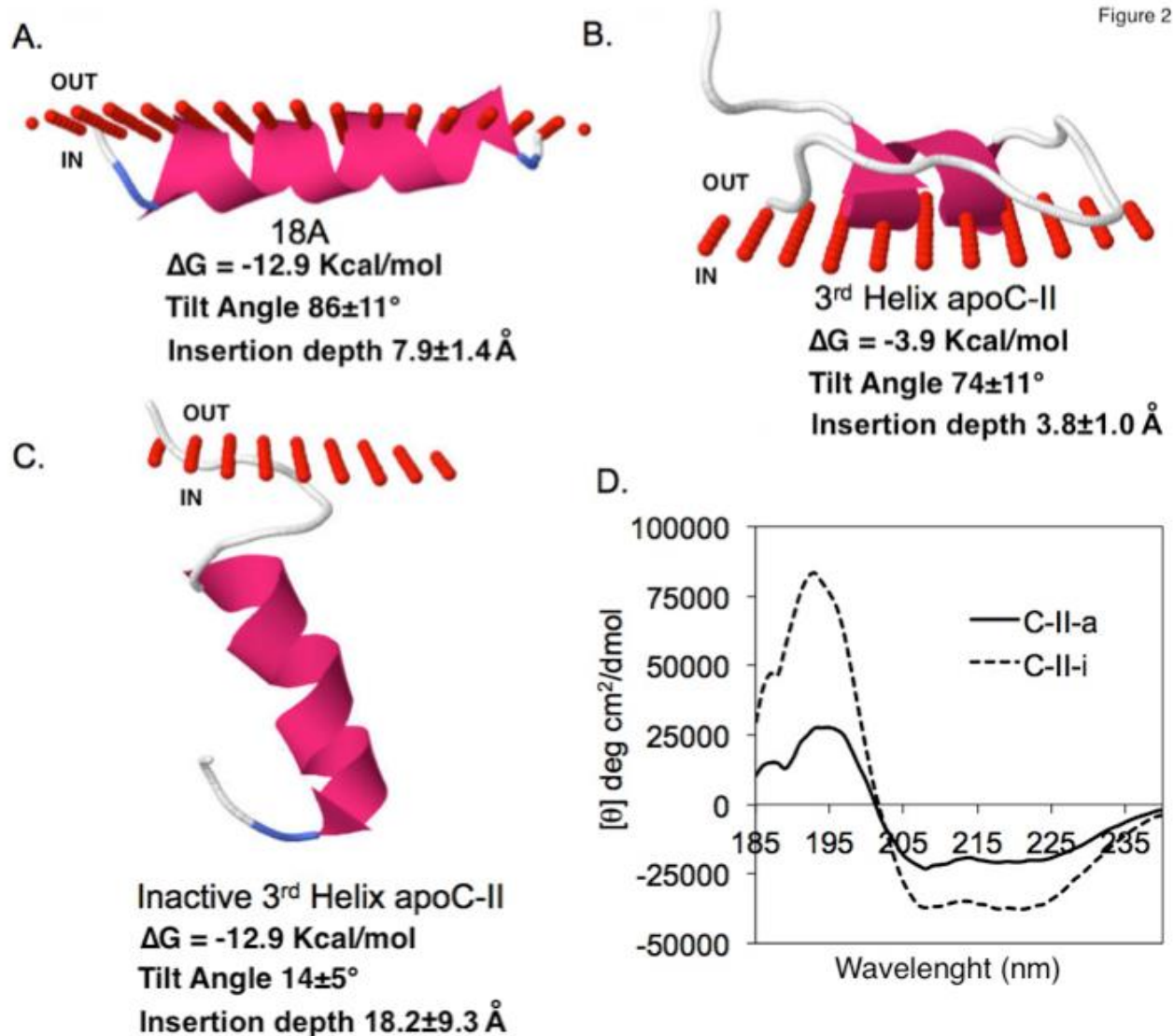
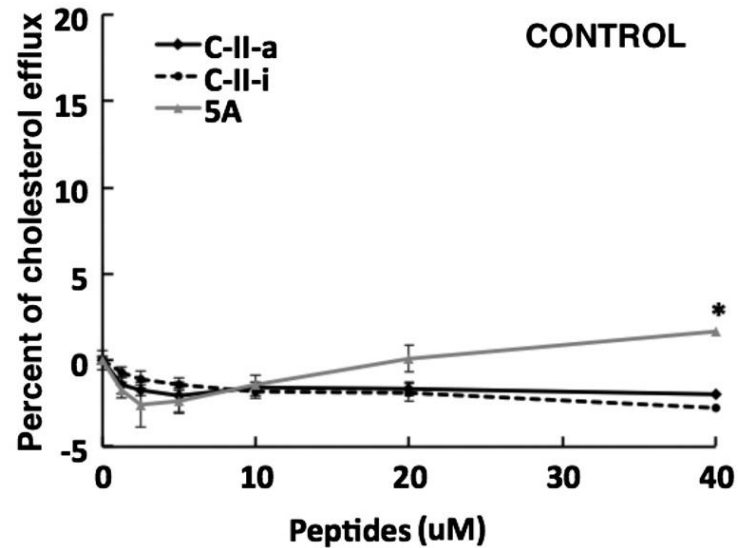
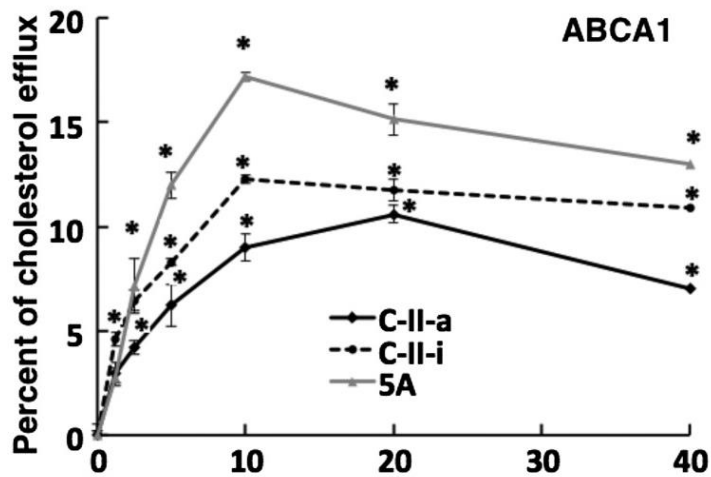
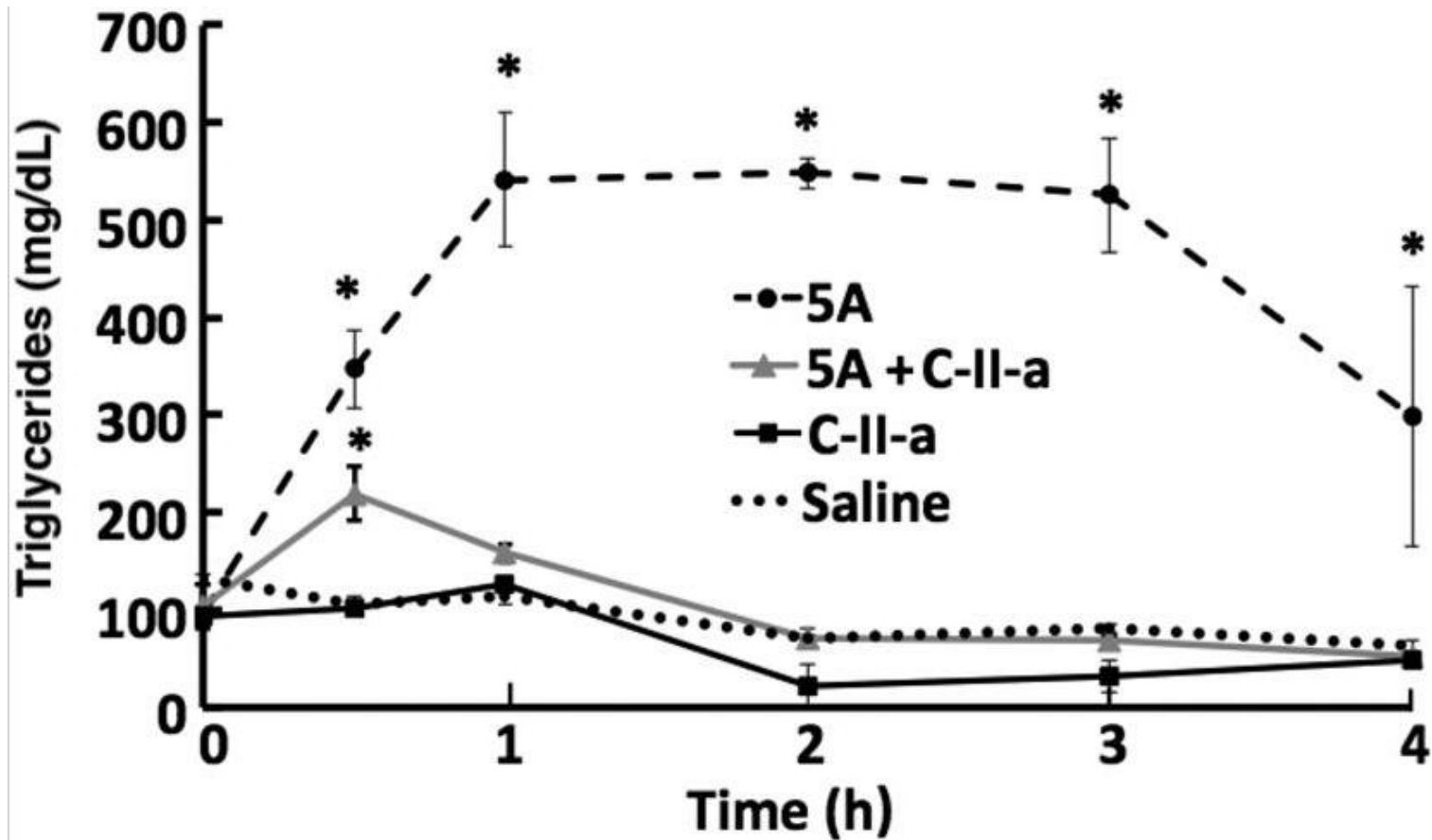


Figure 2

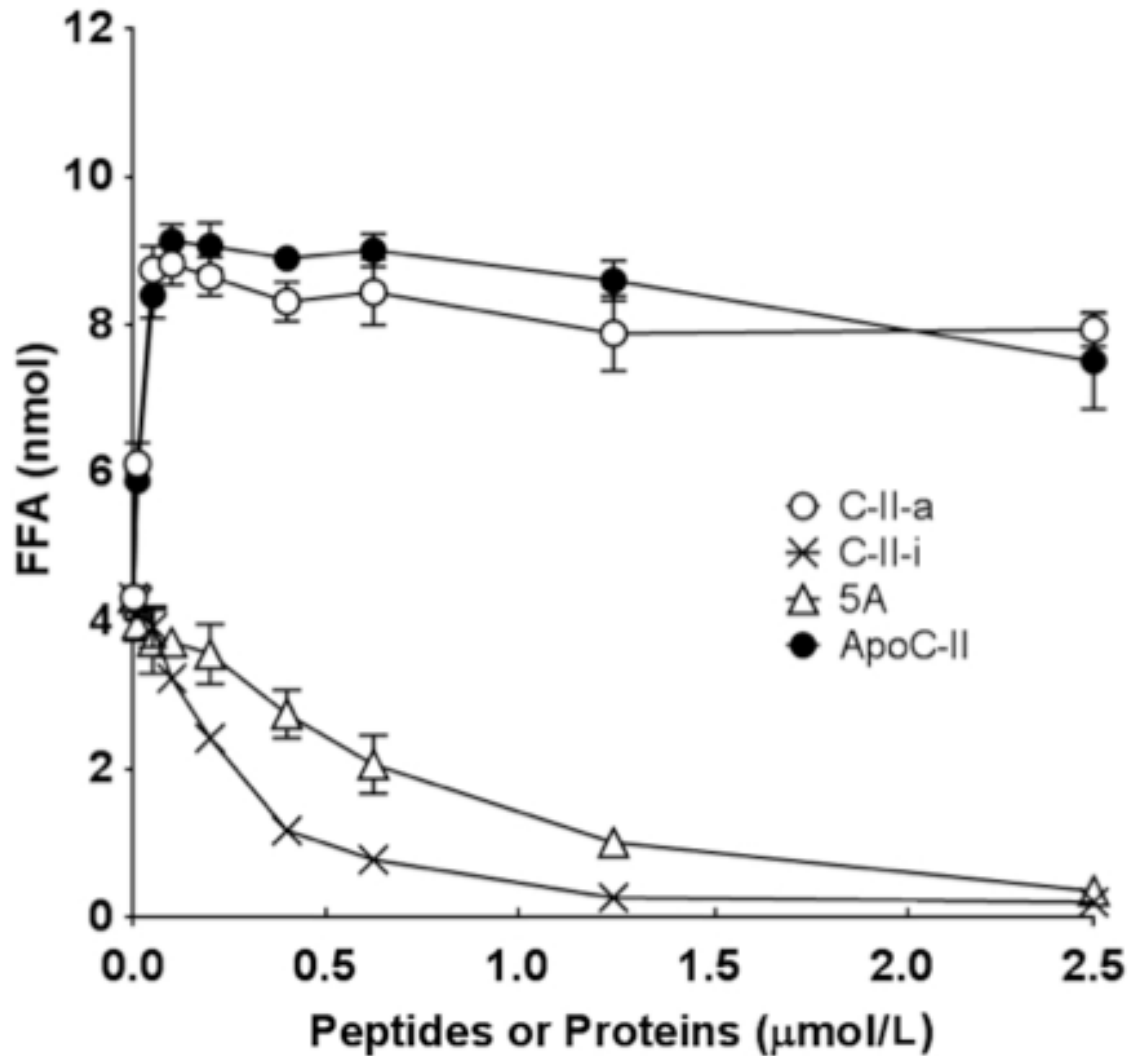
CII-a and CII-i Stimulate Cholesterol Efflux



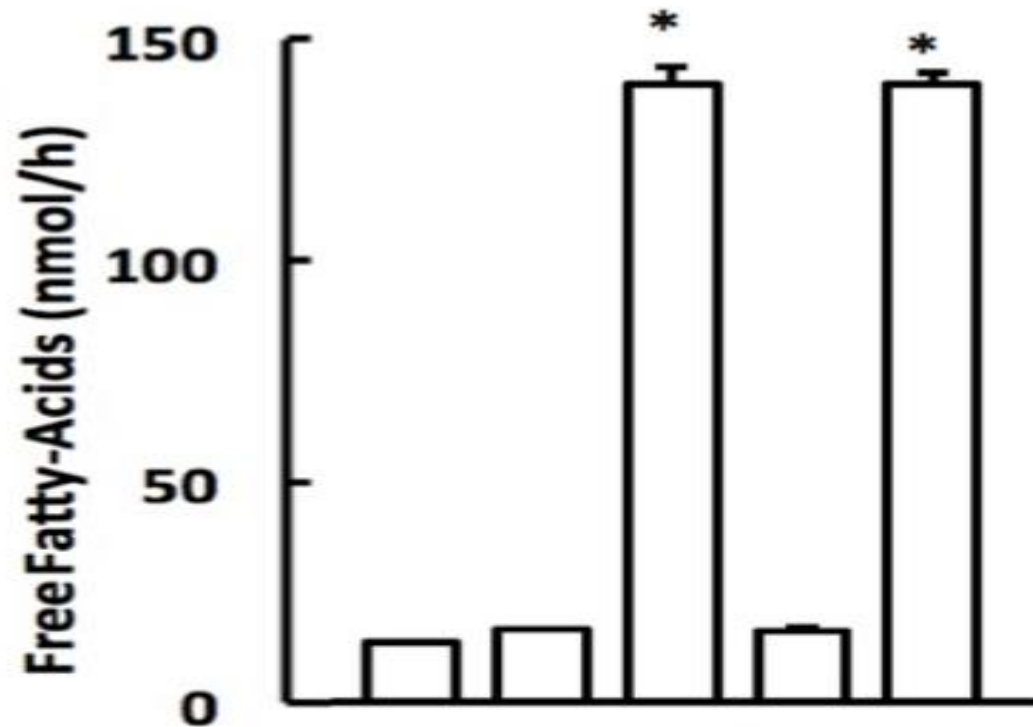
CI-a Prevents Hypertriglyceridemia of 5A



CII-a Activates LPL



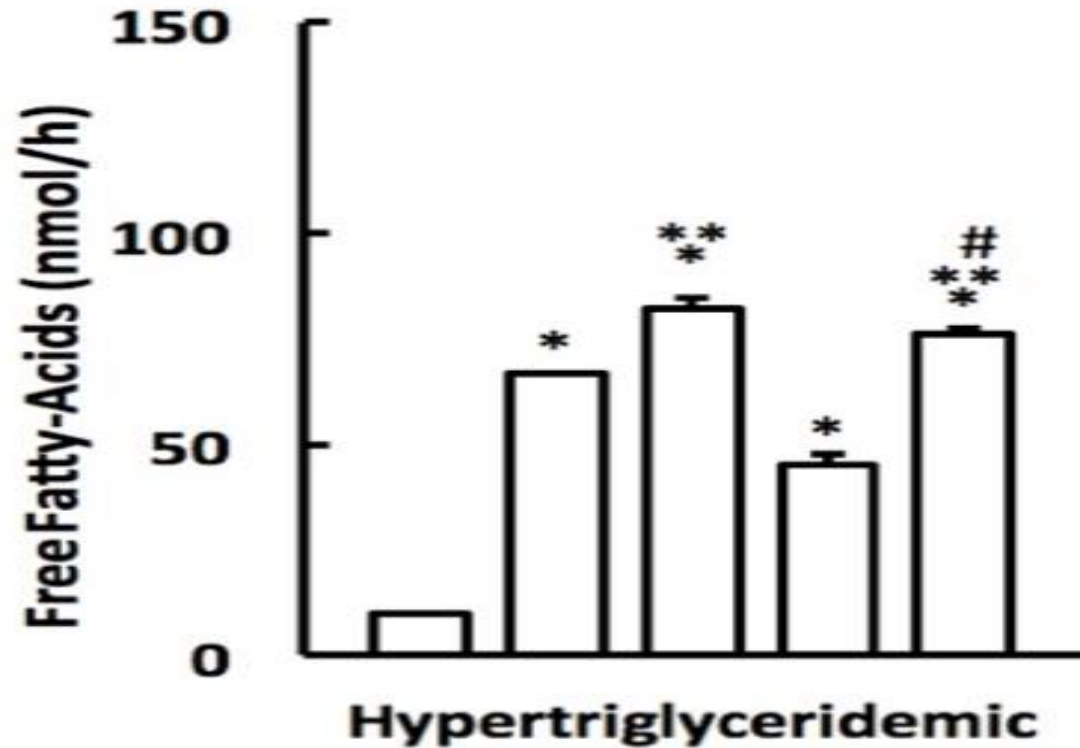
CII-a Restores Lipolysis in apoC-II Deficient Serum



ApoC-II deficient

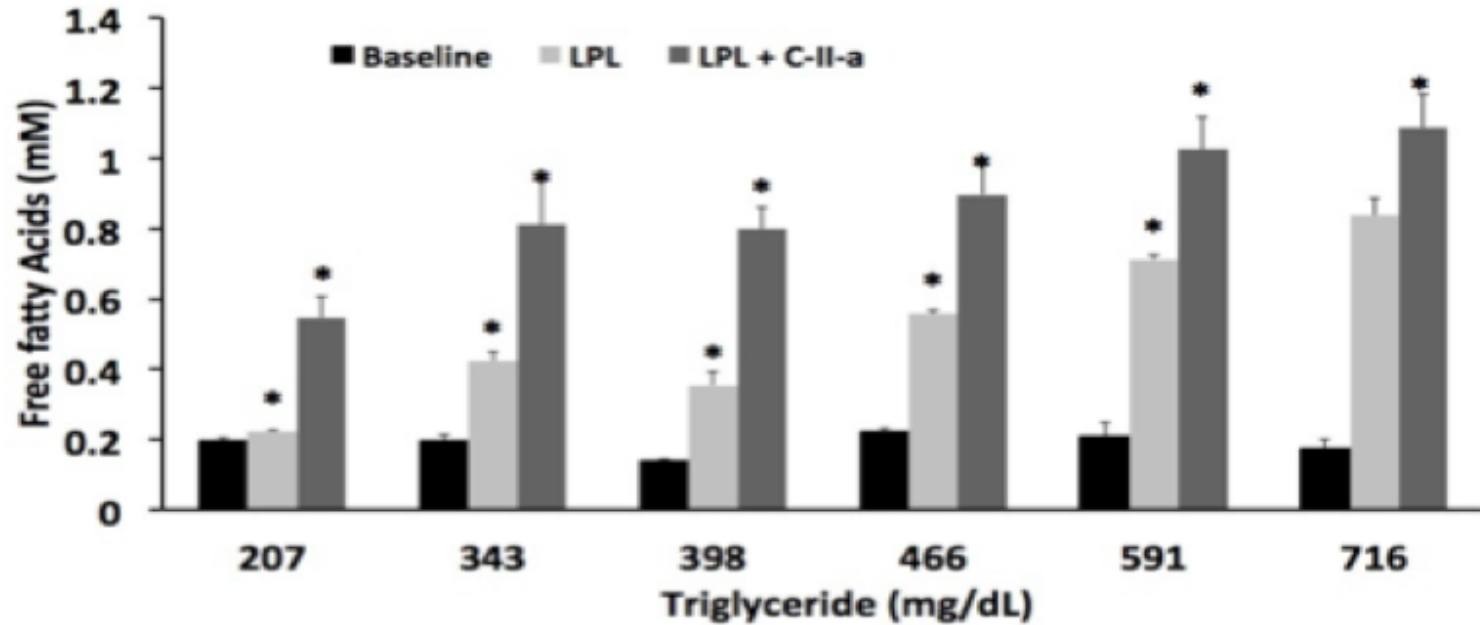
Serum	+	+	+	+	+
LPL	-	+	+	+	+
C-II-a	-	-	+	-	-
hApoC-II	-	-	-	-	+
C-II-i	-	-	-	+	-

CI-a Enhances Lipolysis in HyperTG Serum

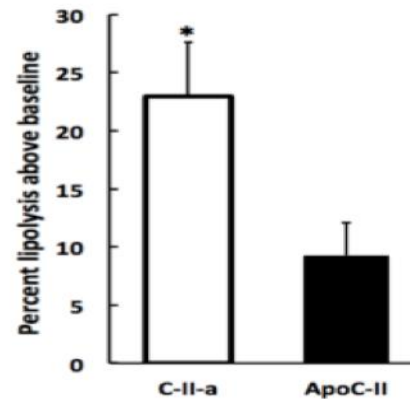
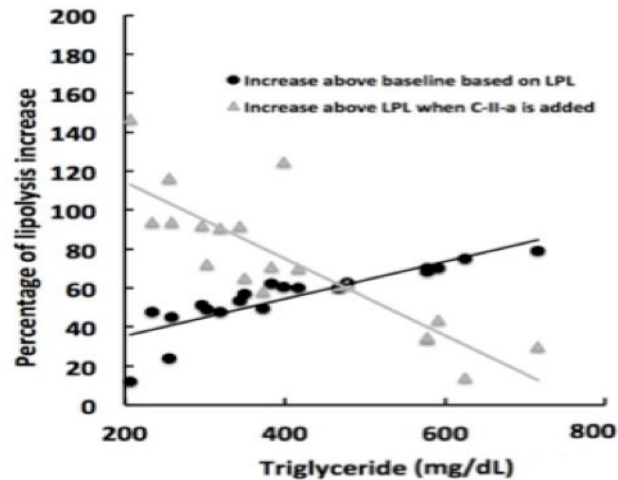
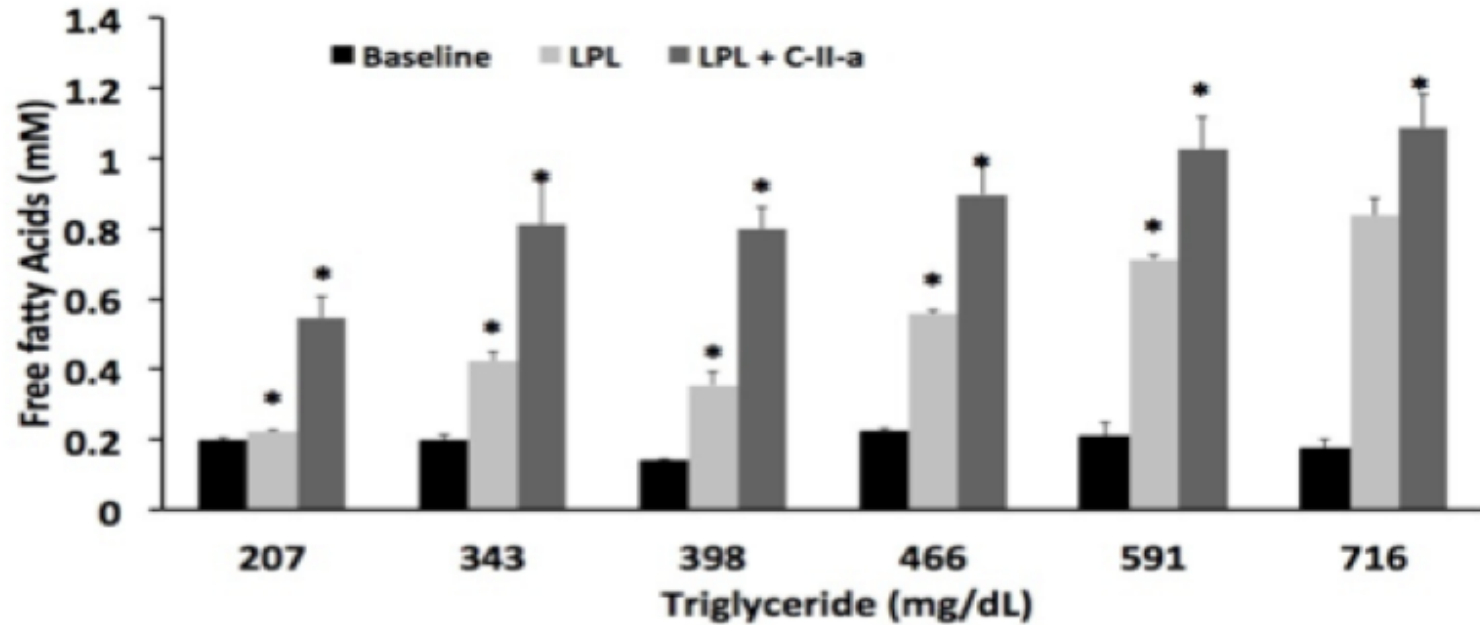


Serum	+	+	+	+	+
LPL	-	+	+	+	+
C-II-a	-	-	+	-	-
hApoC-II	-	-	-	-	+
C-II-i	-	-	-	+	-

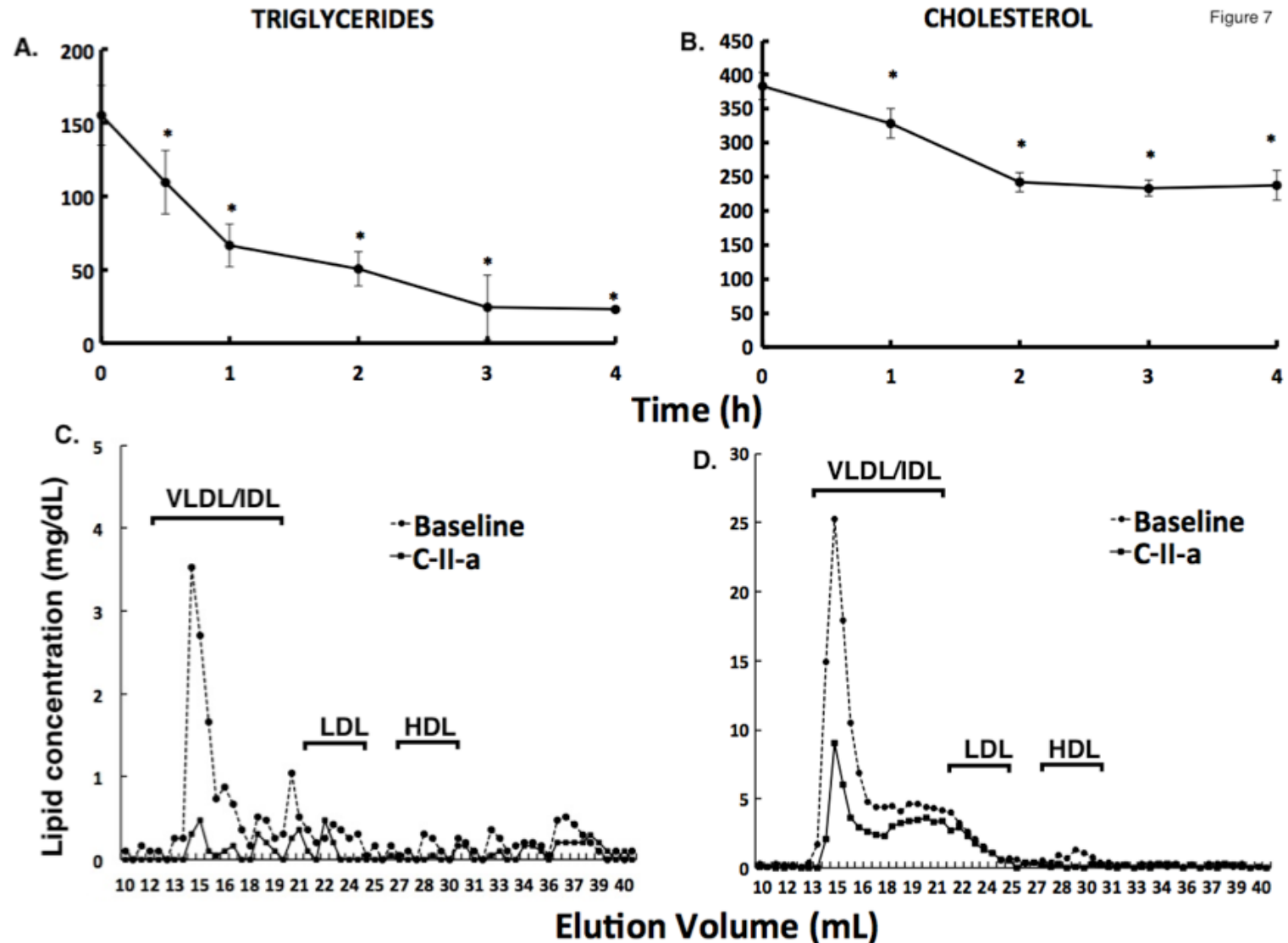
CII-a Increases Lipolysis in HyperTG Serum



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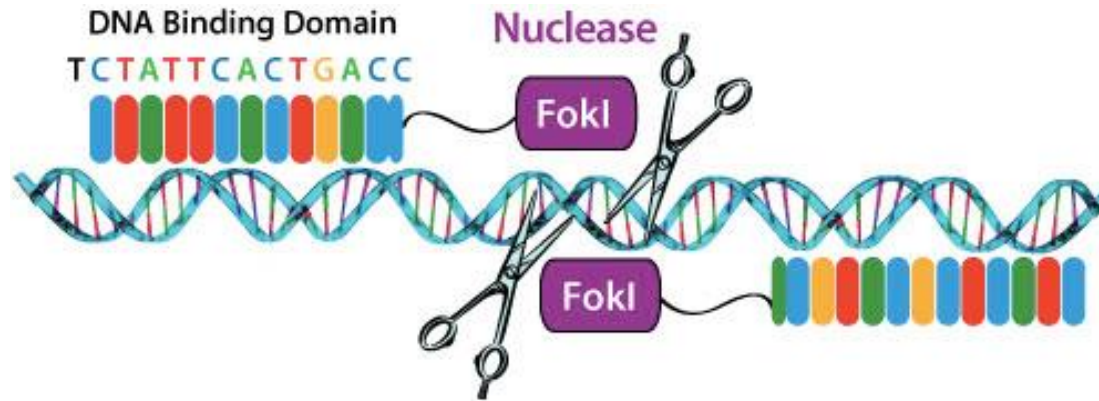
CII-a Increases Lipolysis in ApoE-KO Mice



Overview

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Targeting Strategy of Zn Finger Nuclease



DNA Deletion Site:

Wild type TCCCTCAGAGGTCCAG **gggaaccag** GAAGATGACTCGGGC.....

9-bp deletion TCCCTCAGAGGTCCAG - - - - - GAAGATGACTCGGGC.....

Protein Deletion Site:

Signal site peptide cleavage



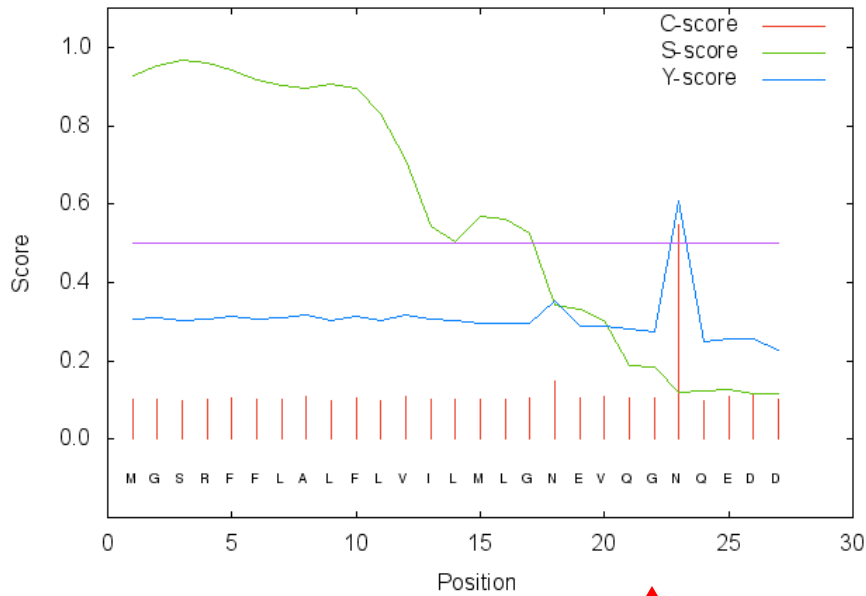
Wild type VQ**GNQ**EDDSGSLALLGTVQGSLLSYWTSAKEVAKDLYQKTYPIISMDEK.....E79

3-aa deletion VQ - - - EDDSGSLALLGTVQGSLLSYWTSAKEVAKDLYQKTYPIISMDEK.....E79

Deletion Predicted to Prevent Signal Peptide Cleavage

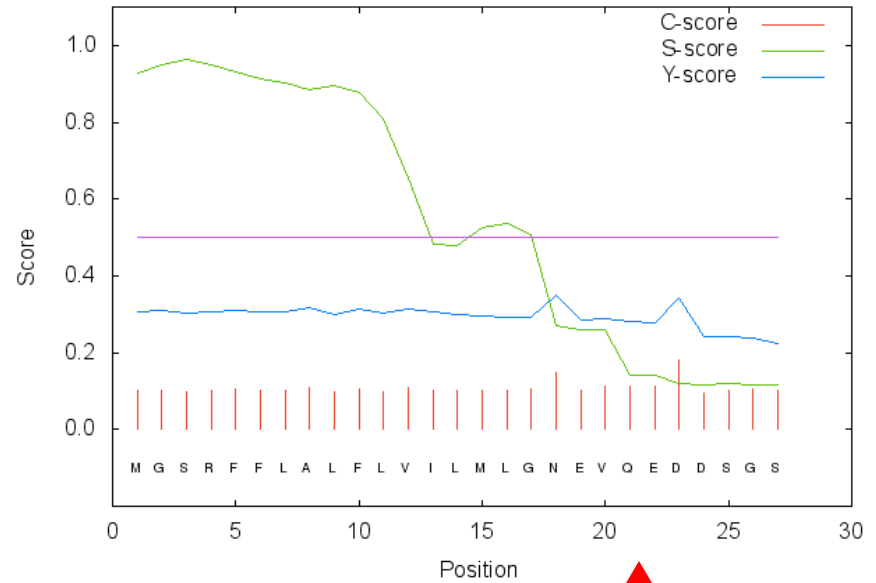
Wild Type Sequence

SignalP-4.1 prediction (euk networks): Sequence

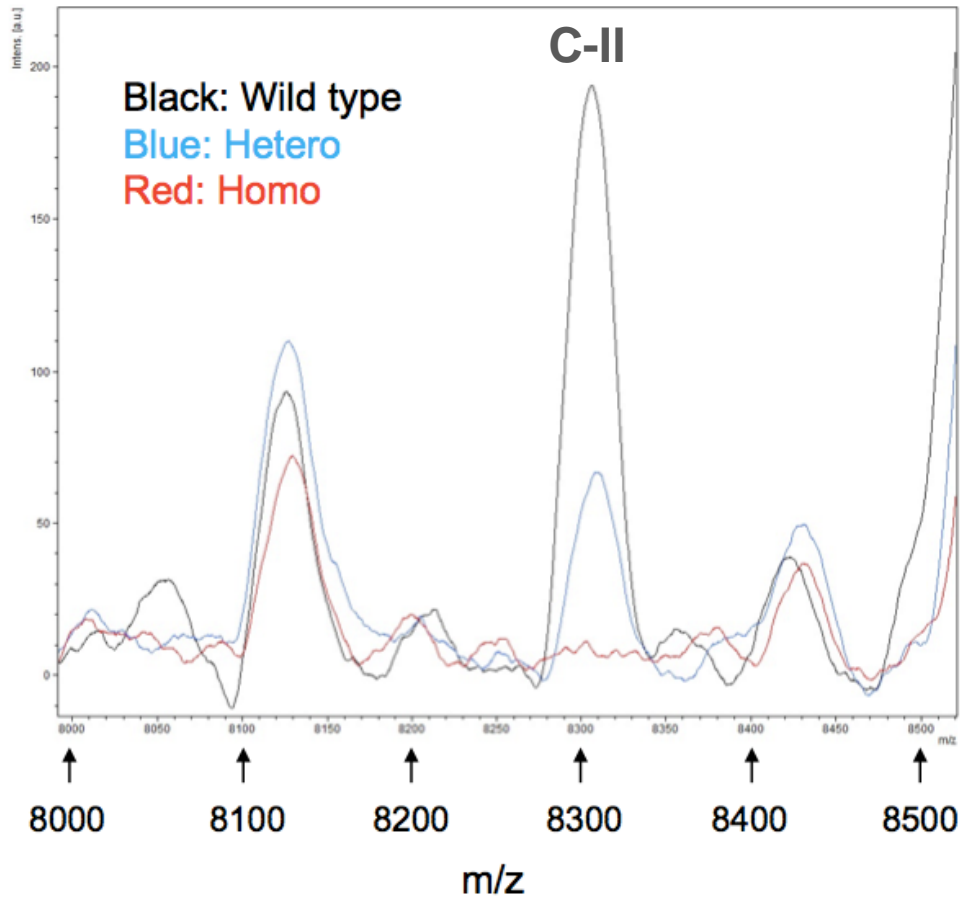


Deletion Sequence

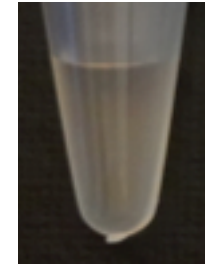
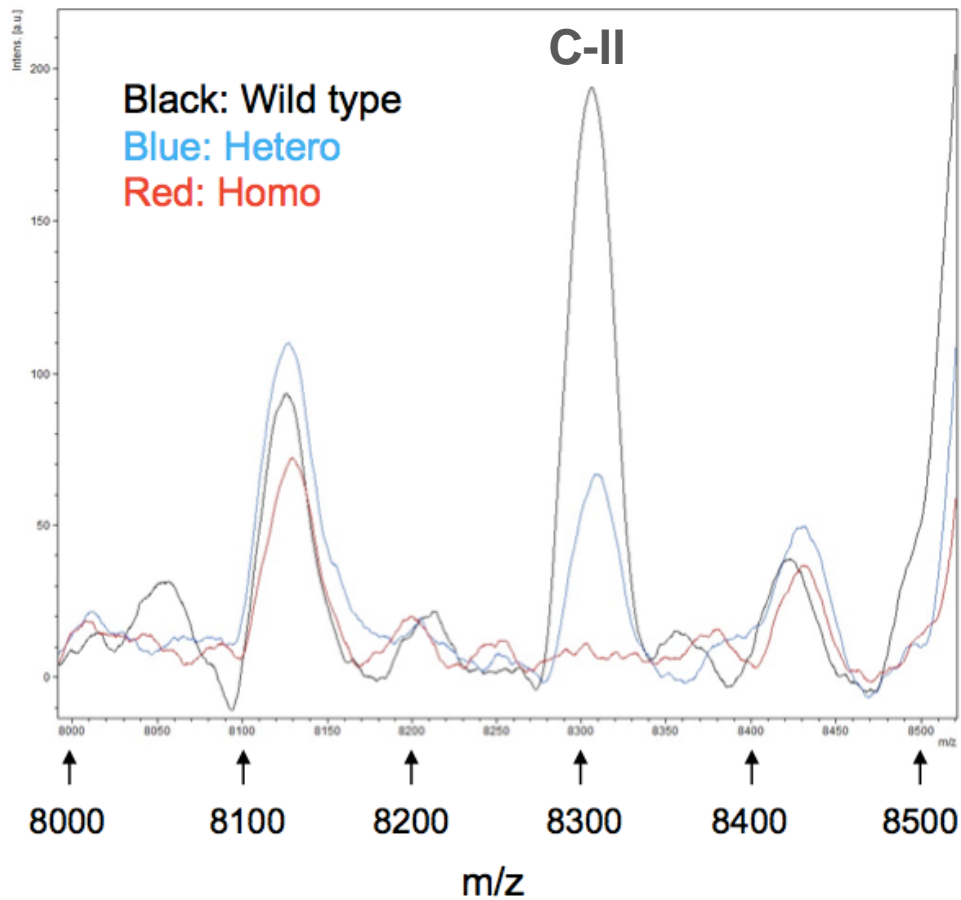
SignalP-4.1 prediction (euk networks): Sequence



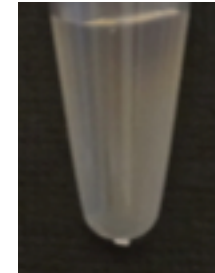
MALDI-TOF Analysis of ApoC-II



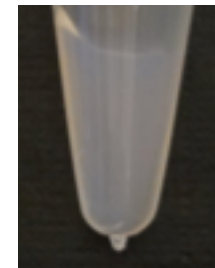
MALDI-TOF Analysis of ApoC-II



WT

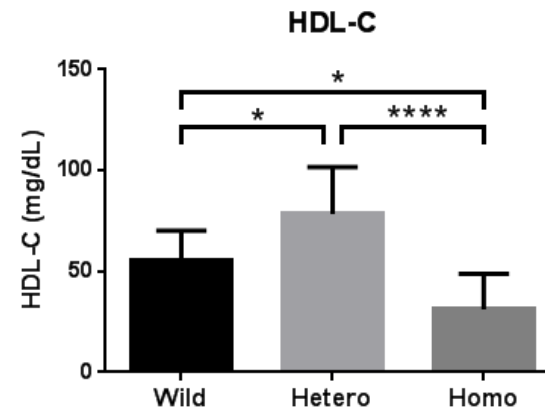
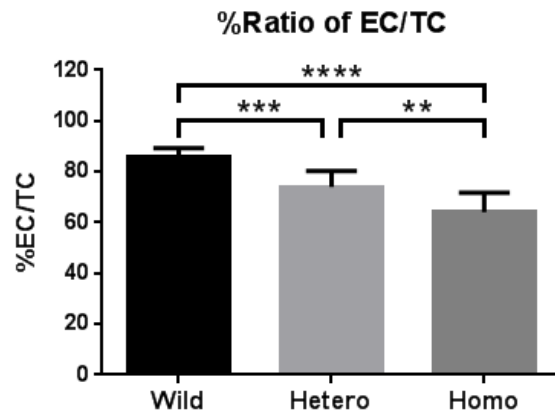
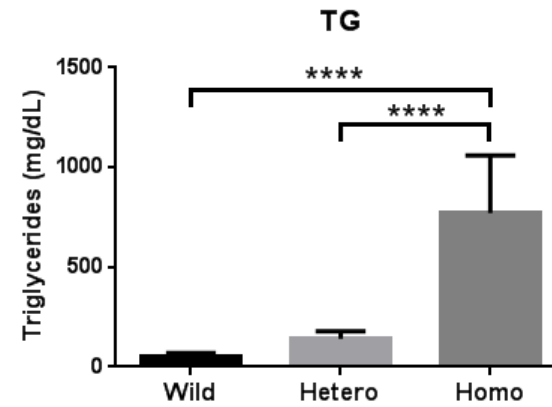
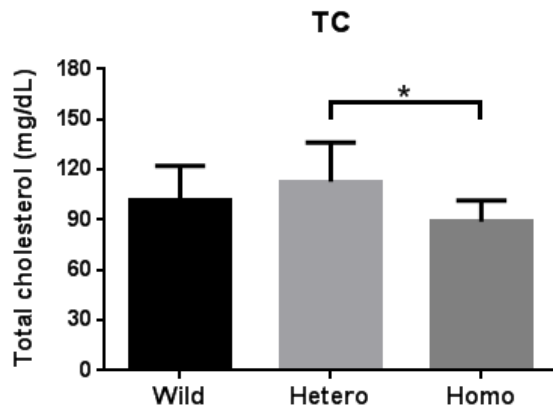


Hetero

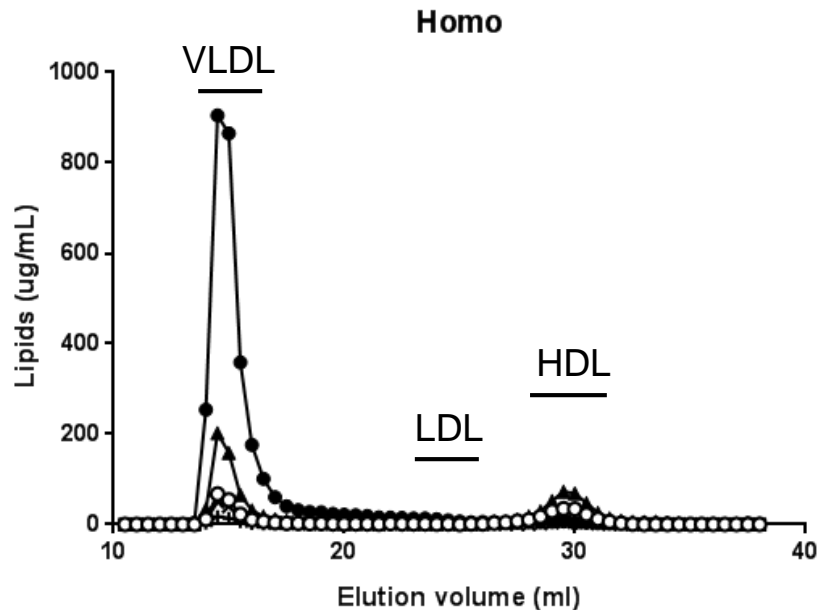
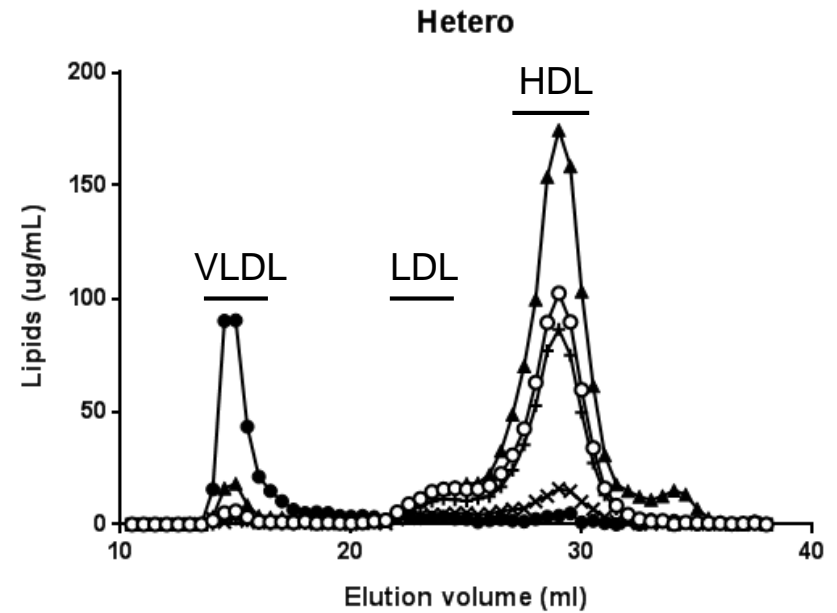
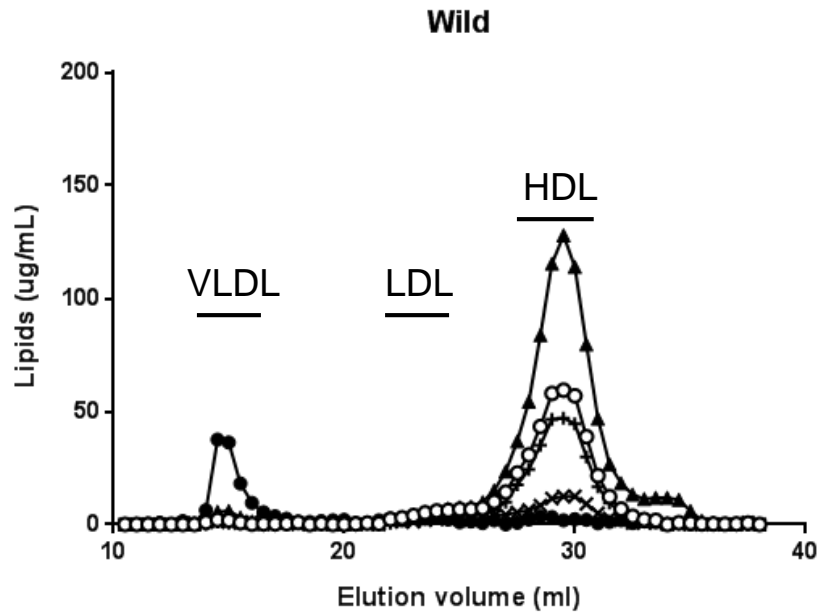


Homo

Plasma Lipid Levels in apoCII-KO mice

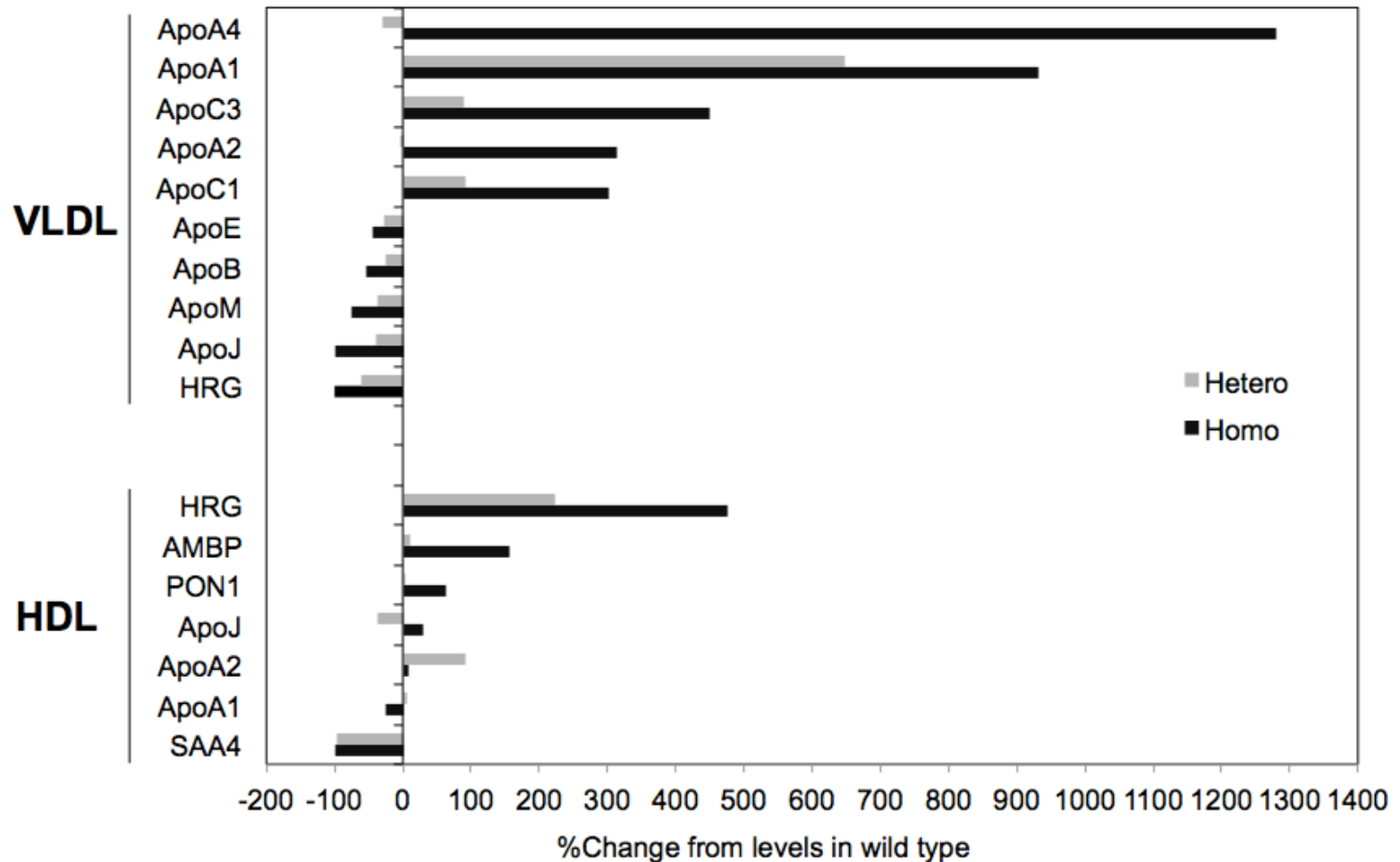


Lipoprotein profiles in apoCII-KO mice



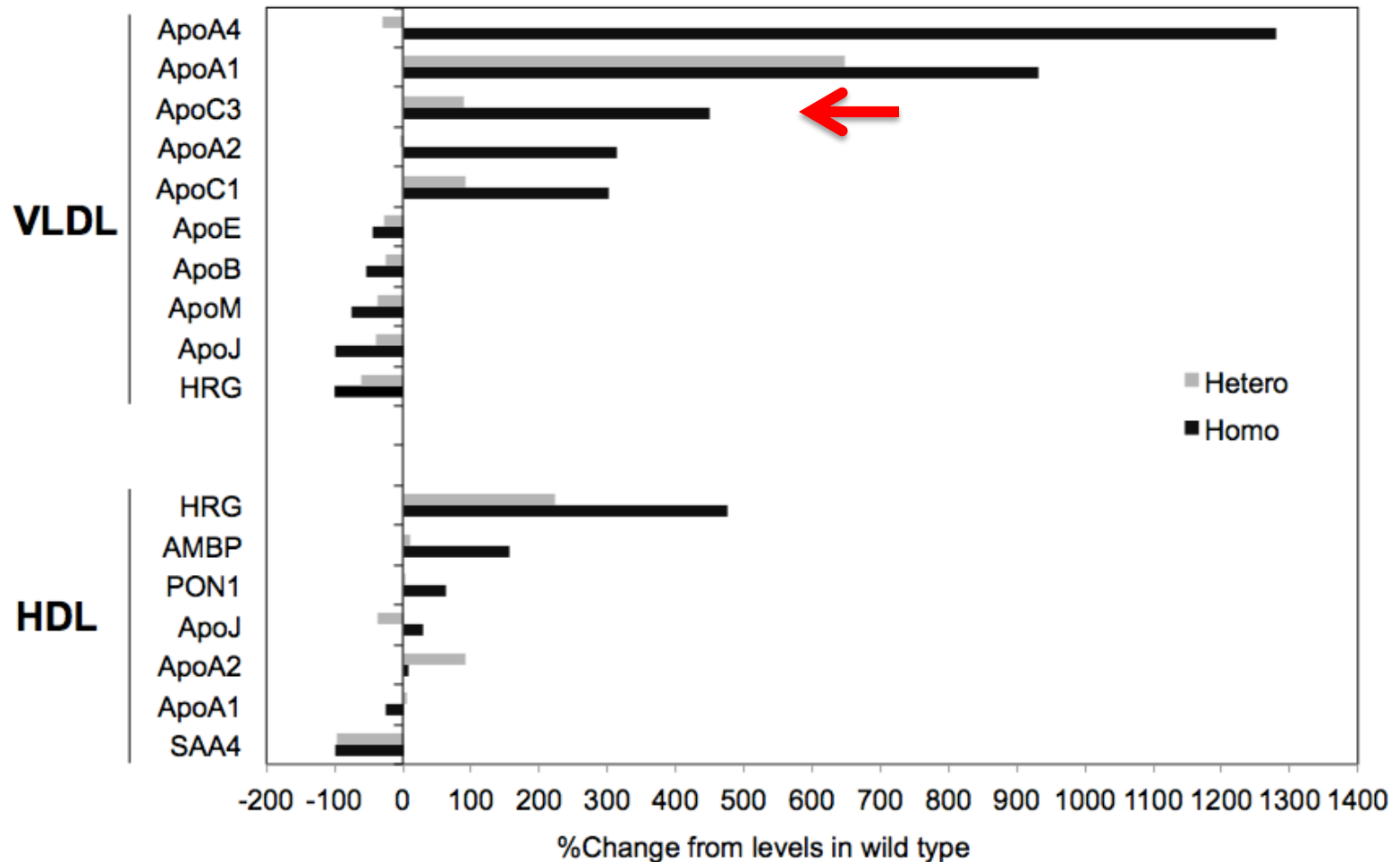
- TC (ug/ml)
- TG (ug/ml)
- ▲ PL (ug/ml)
- * FC (ug/ml)
- + CE (ug/ml)

Proteome Content of Lipoproteins



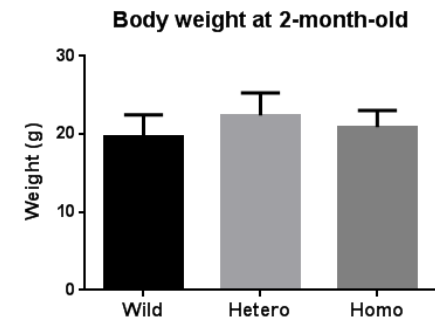
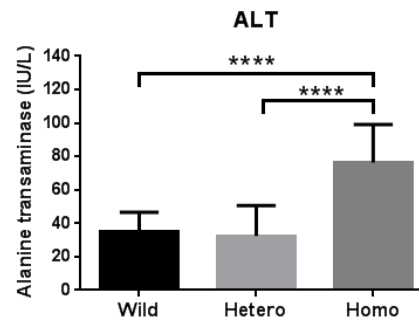
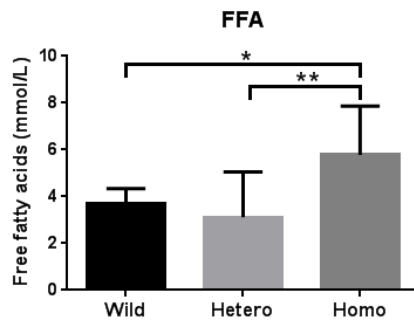
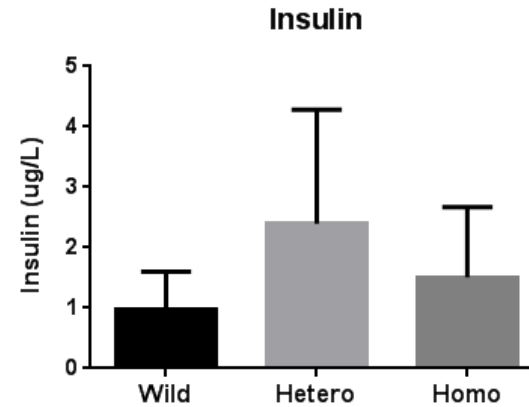
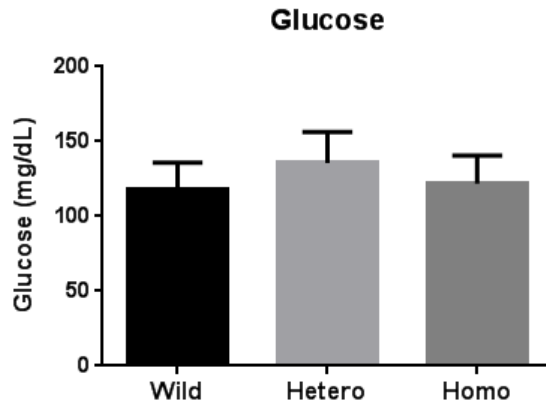
HRG, Histidine-rich glycoprotein; AMBP, Alpha-1-microglobulin/bikunin precursor; PON1, Paraoxonase 1; SAA4, Serum amyloid A4.

Proteome Content of Lipoproteins



HRG, Histidine-rich glycoprotein; AMBP, Alpha-1-microglobulin/bikunin precursor; PON1, Paraoxonase 1; SAA4, Serum amyloid A4.

Metabolic Parameters of ApoC-II KO mice (2-mo.)

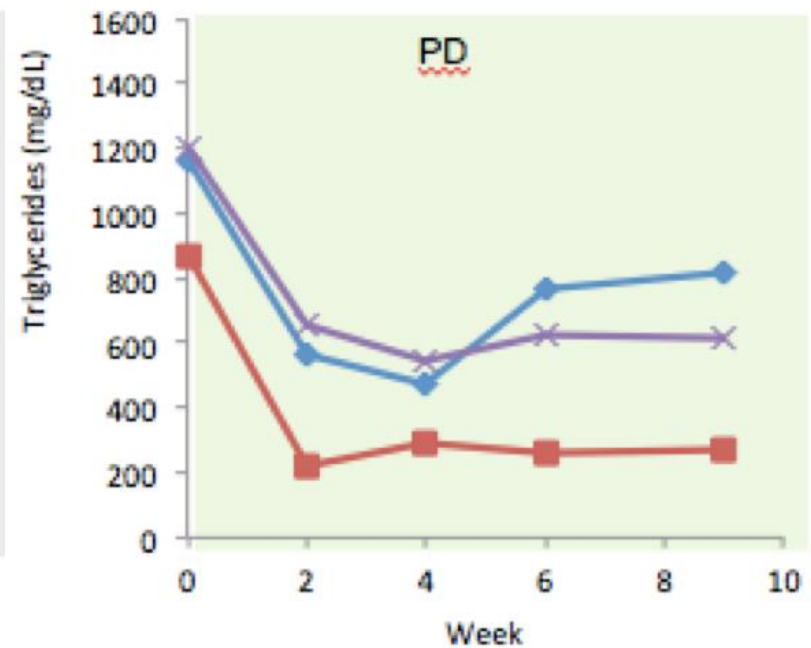
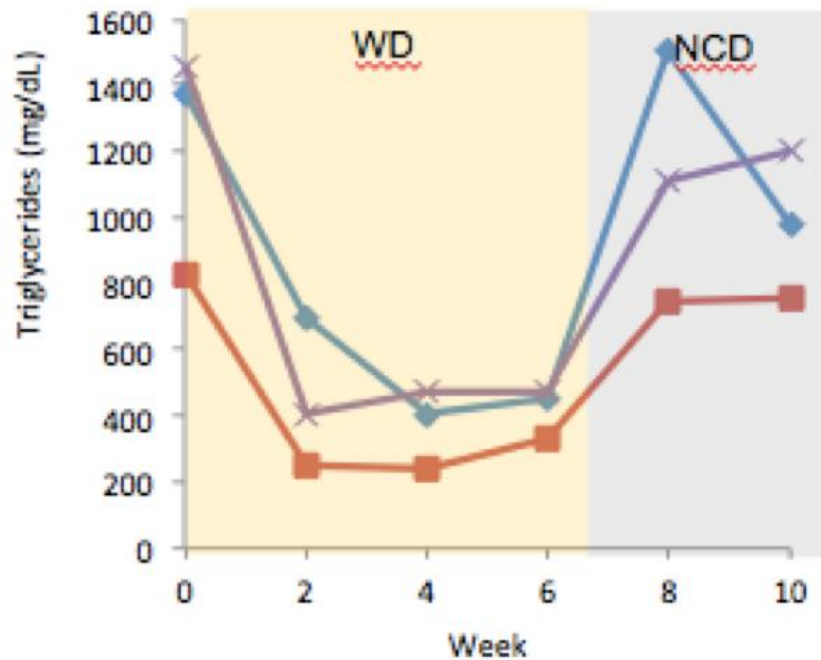


Effect of Diets on Plasma Triglycerides

Diet Timing

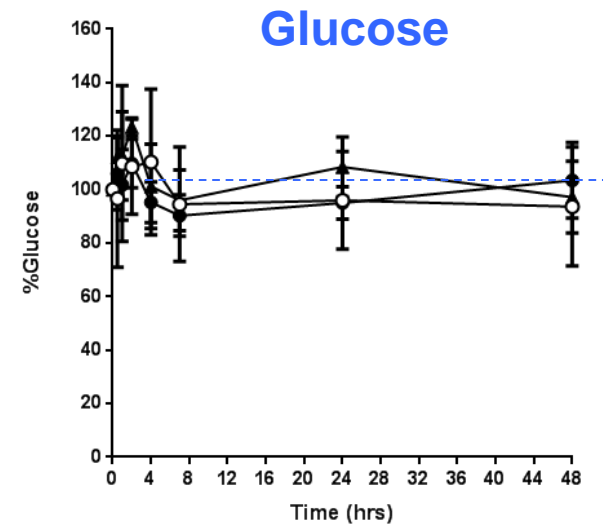
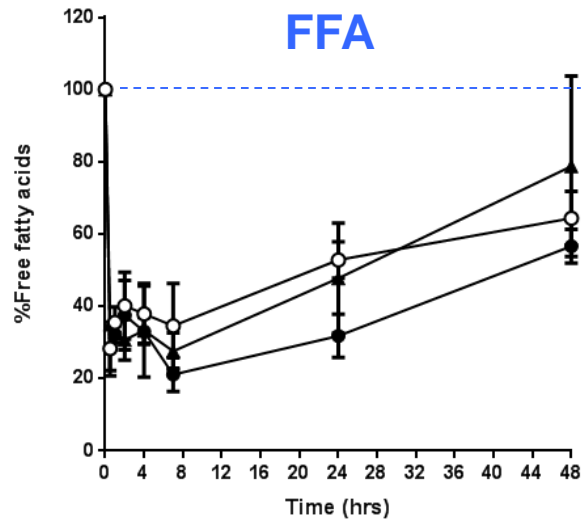
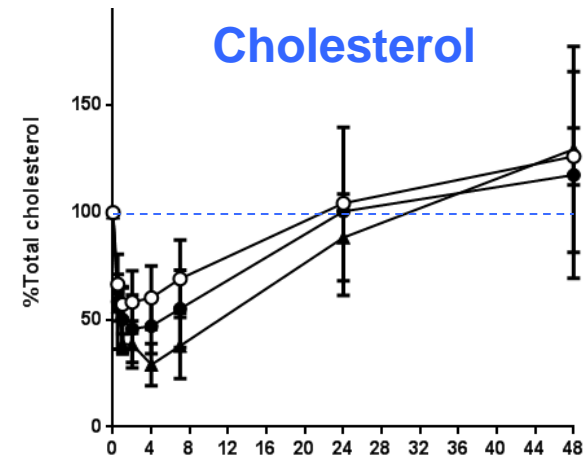
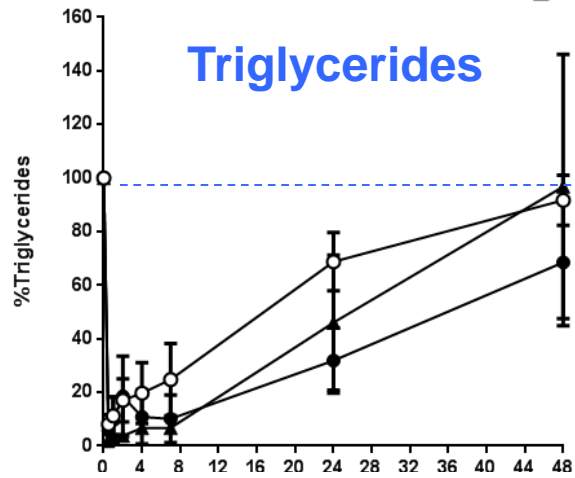
NCD → WD → NCD

NCD → PD



Effect of C-IIa on Lipids in ApoC-II KO Mice

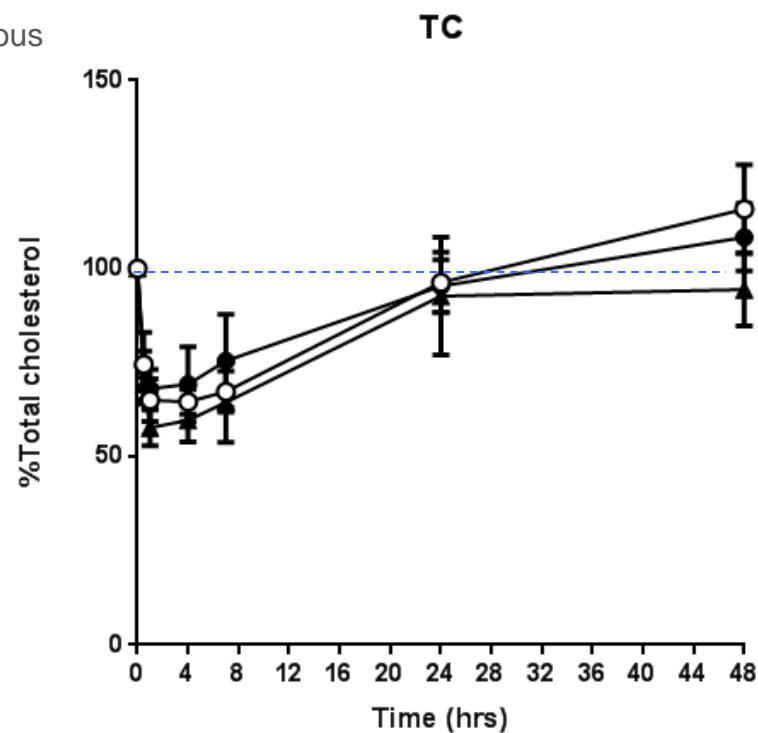
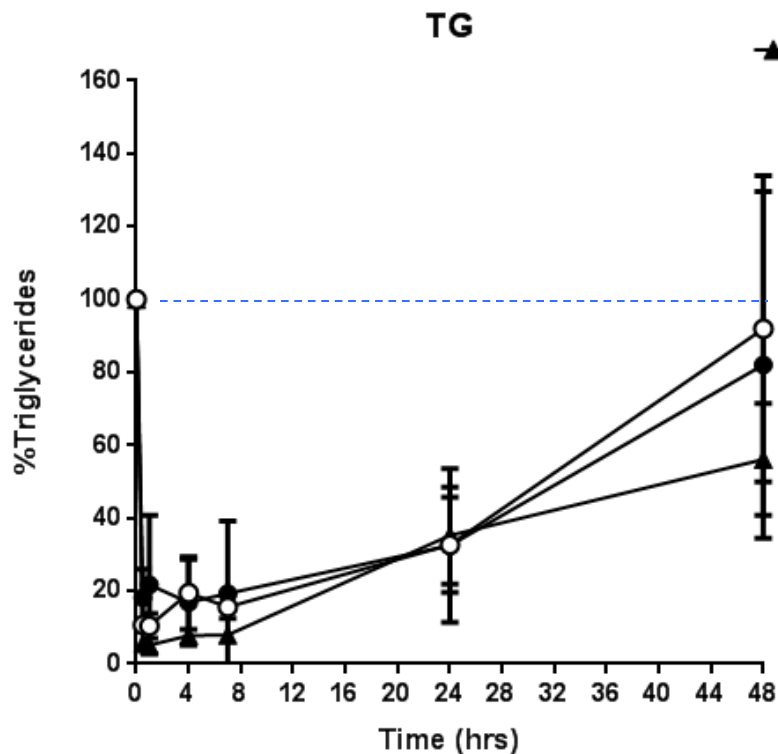
- C-II-a: 0.91 mg/kg (0.2 $\mu\text{mol/kg}$)
- C-II-a: 4.6 mg/kg (1.0 $\mu\text{mol/kg}$)
- ▲ C-II-a: 22.9 mg/kg (5 $\mu\text{mol/kg}$)



SQ and IM Administration Similar PK/PD to IV

C-IIa Injection (1 umol/kg)

- Subcutaneous
- Intramuscular
- ▲ Intravenous



Summary

- Described a novel apoC-II mimetic peptide (C-IIa) that activates LPL
- Promotes ABCA1-dependent cholesterol efflux
- Restores *in vitro* lipolysis of human ApoC-II deficient serum
- Lowers TG in other forms of hypertriglyceridemia
- Corrects lipid abnormalities in apoC-II KO mice
- C-IIa may have value as a therapy for apoC-II deficiency and other forms of hypertriglyceridemia and diabetes