

Novel TLR7 and TLR9 Dual Antagonist Lowers Cholesterol in Hyperlipidemic Mice Through IL-10-Mediated Activation of LXR and Increased Fecal Neutral Sterol Loss.

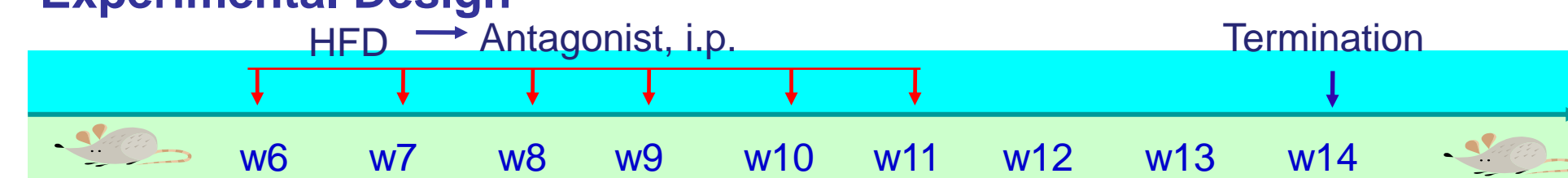
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INTRODUCTION

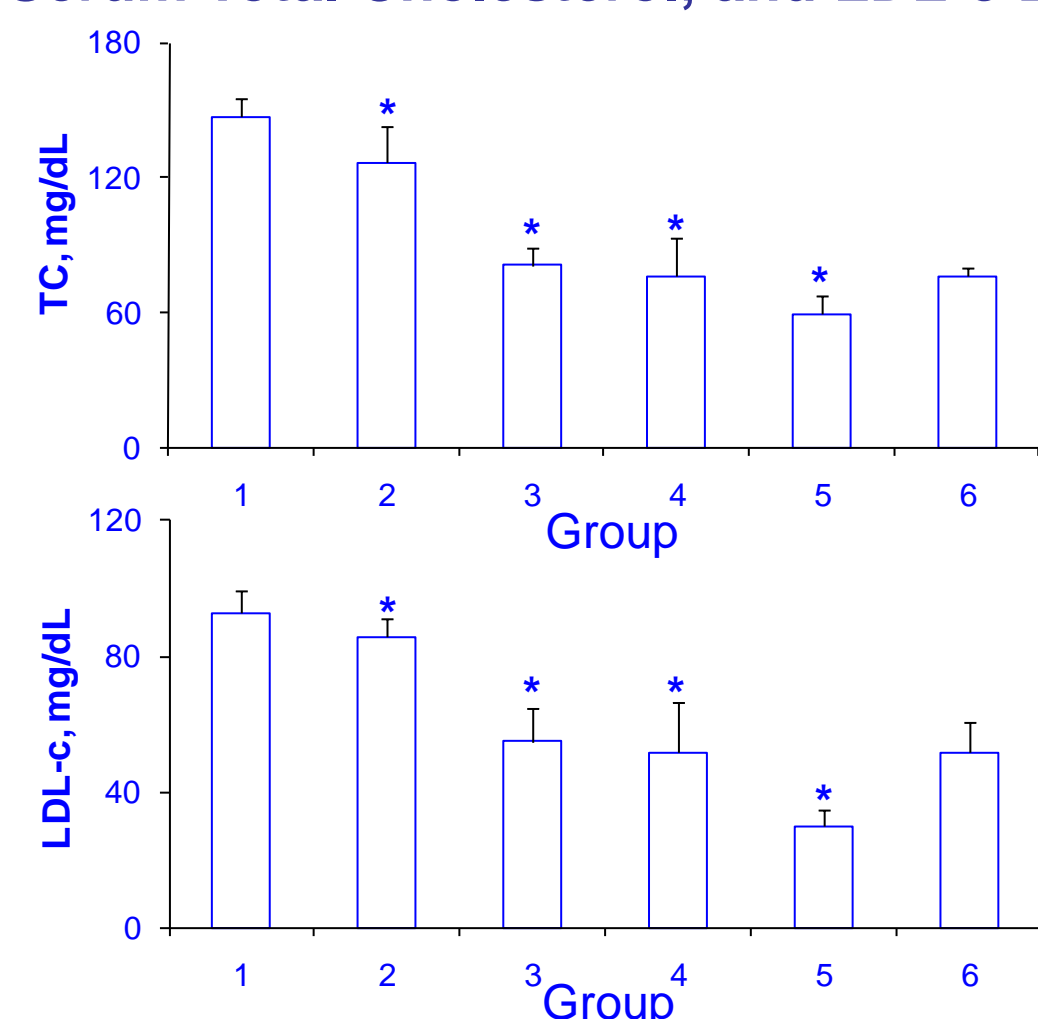
- Hyperlipidemia is characterized by abnormal or elevated lipid and/or lipoprotein levels in the blood.
- Elevated lipid levels have been observed in autoimmune diseases:
 - Lupus, rheumatoid arthritis, psoriasis and others. (*Frostegard J. Arterioscler Thromb Vasc Biol 2005, 25: 1776*)
 - Increase in cardiovascular disease-related morbidity and mortality. (*Sarzi-Puttini, P. et al. Autoimmun Rev 2010, 9: 849*)
- Toll like receptors (TLRs) are involved in inflammation processes that contribute to alteration of lipid levels.
- Our dual antagonist to TLR7 and TLR9 lowers total cholesterol (TC), LDL-cholesterol (LDL-c), liver triglycerides and leptin in a dose-dependent manner in C57BL/6 and ApoE^{-/-} mice fed with a high fat diet (HFD).
 - Serum levels of TC were inversely correlated with levels of IL-10.
 - Antagonist treatment was associated with inhibition of plaque formation and improvement of liver and kidney steatosis.
- In the present study, we have analyzed gene expression changes in the liver and intestine of mice fed HFD and treated with the antagonist and evaluated the impact of IL-10 neutralization on TC lowering.

Experimental Design

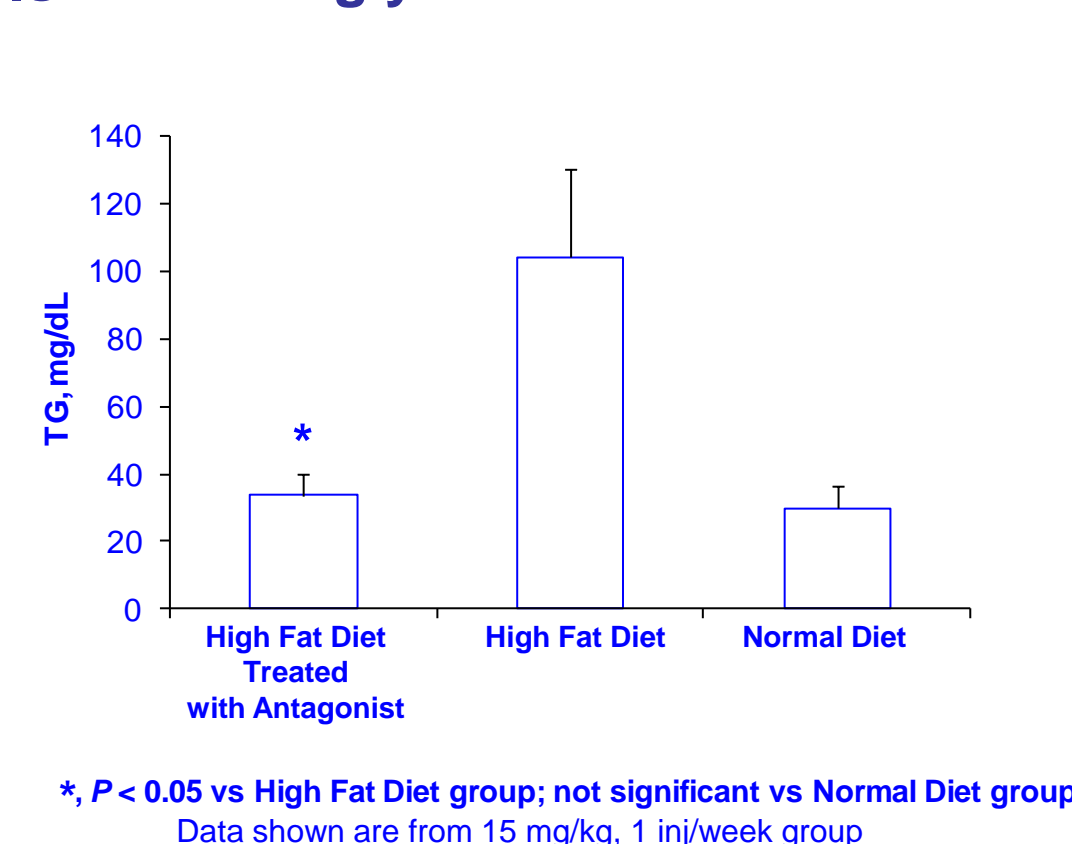


- HFD feeding started at week 6 and continued until the end of the study (week 14).
- Mice were divided into the following groups (N = 10):
 - Group 1: High Fat Diet (HFD)
 - Group 2: HFD + Antagonist 3 mg/kg, 2 inj/week
 - Group 3: HFD + Antagonist 6 mg/kg, 2 inj/week
 - Group 4: HFD + Antagonist 15 mg/kg, 1 inj/week
 - Group 5: HFD + Antagonist 15 mg/kg, 2 inj/week
 - Group 6: Normal Diet

Antagonist Dose-Dependently Decreases Serum Total Cholesterol, and LDL-c Levels

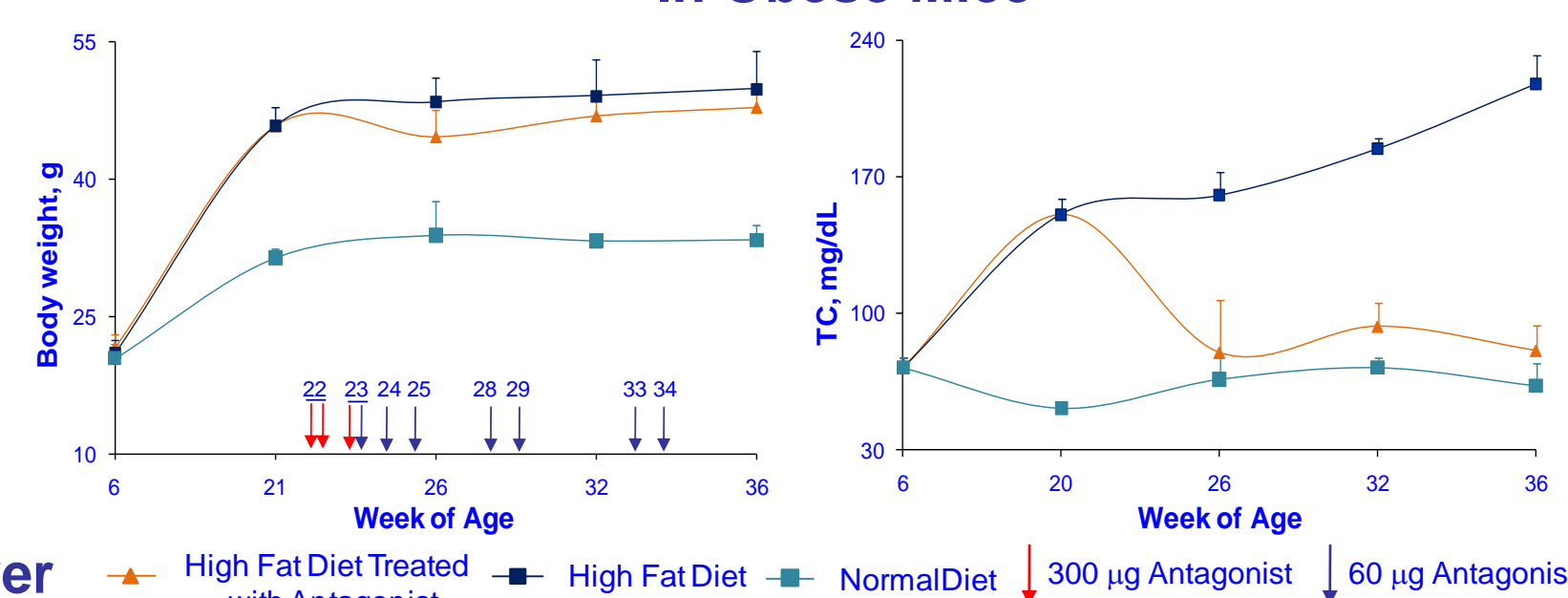


Antagonist Decreases Liver Triglyceride Levels

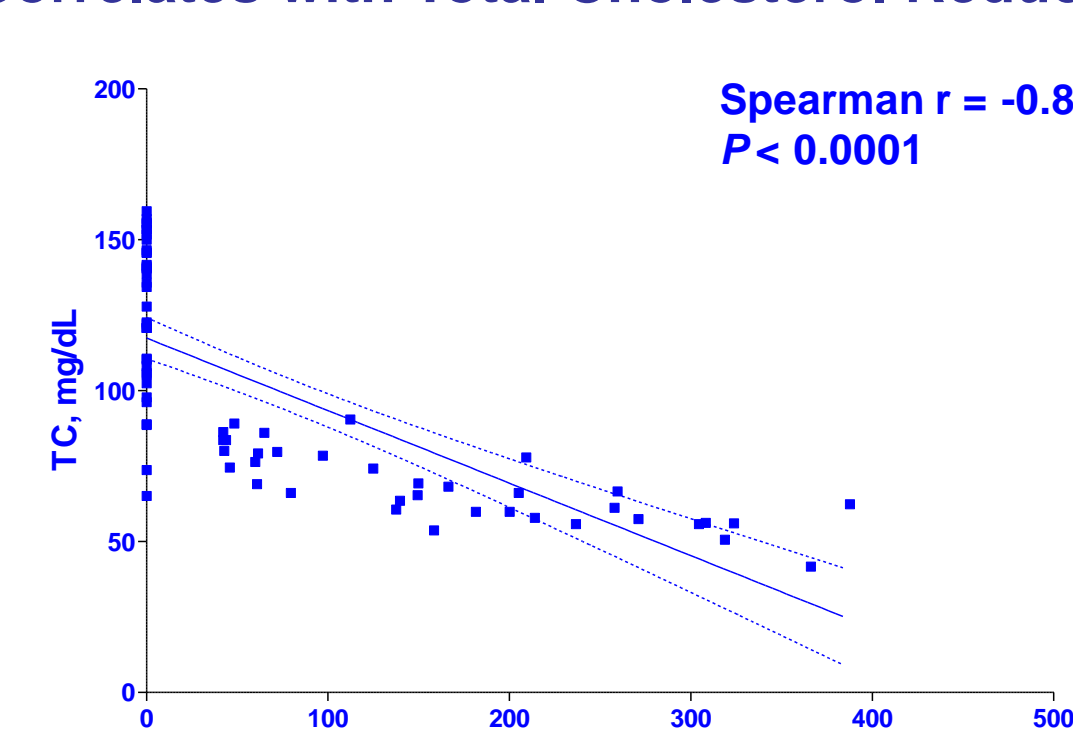


C57BL/6

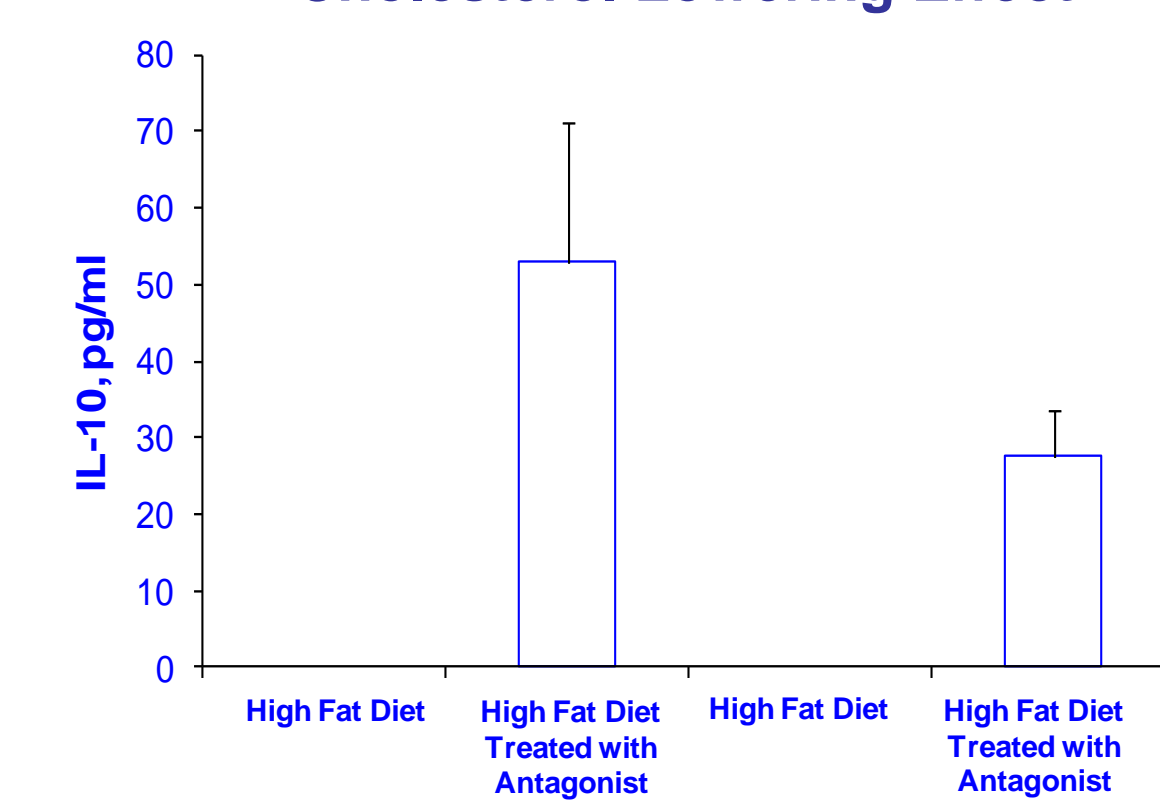
Antagonist Decreases Serum Total Cholesterol In Obese Mice



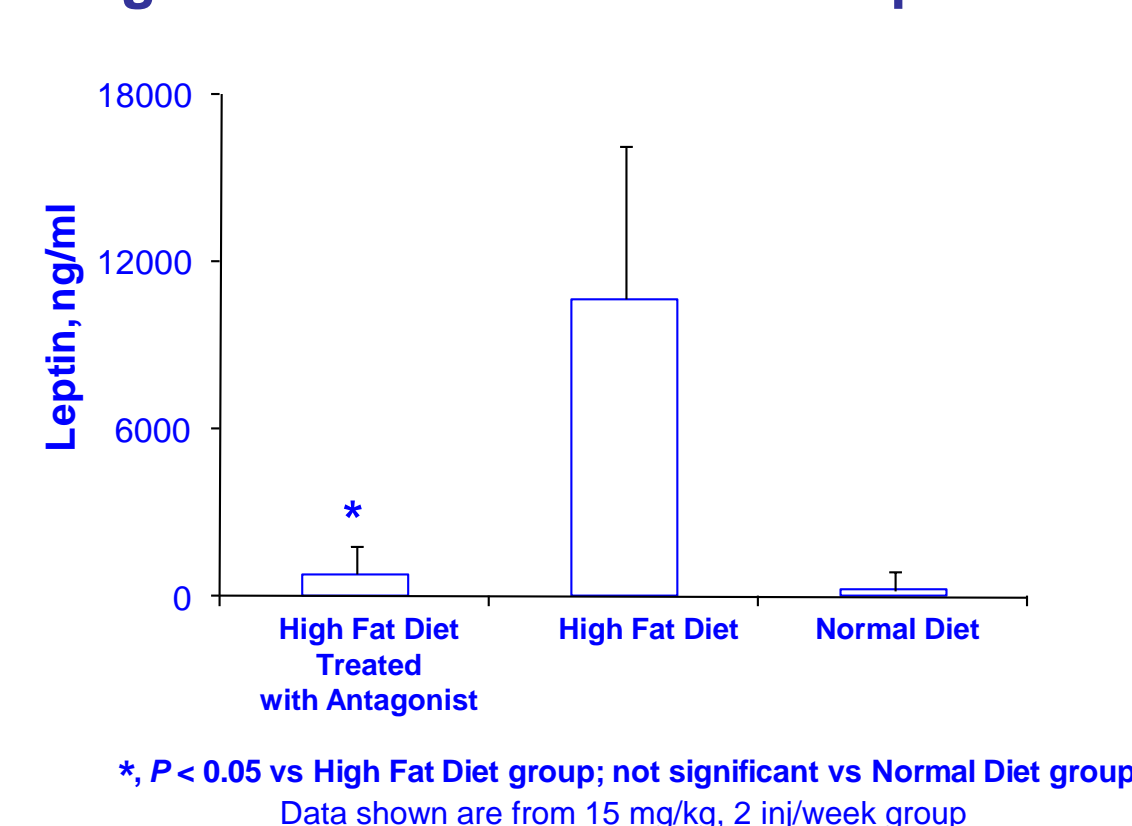
Antagonist-Induced IL-10 Production Correlates with Total Cholesterol Reduction



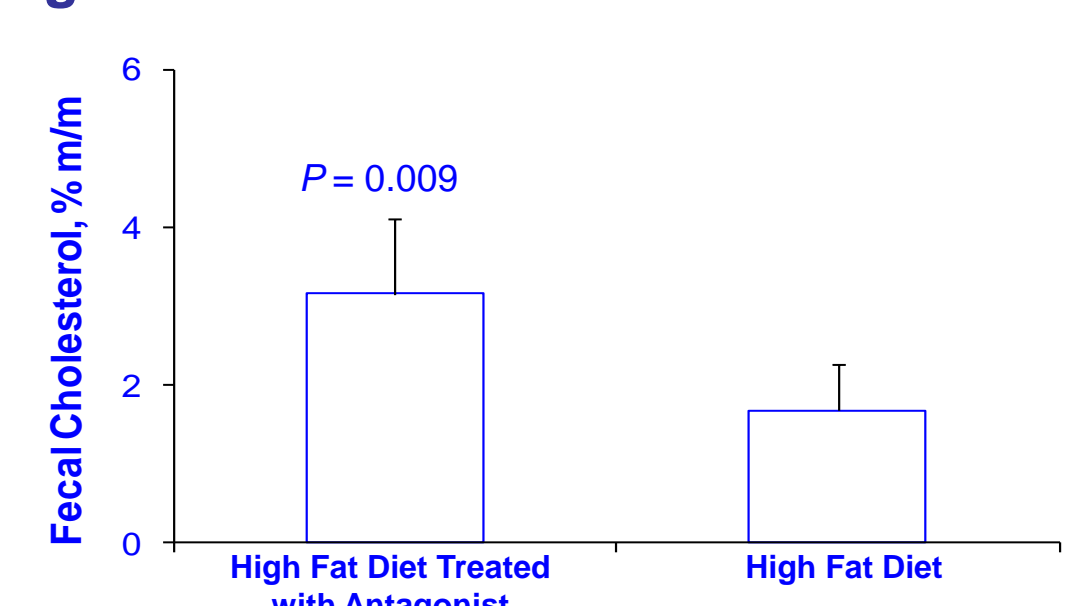
Neutralizing IL-10 Reverses Antagonist-Induced Cholesterol Lowering Effect



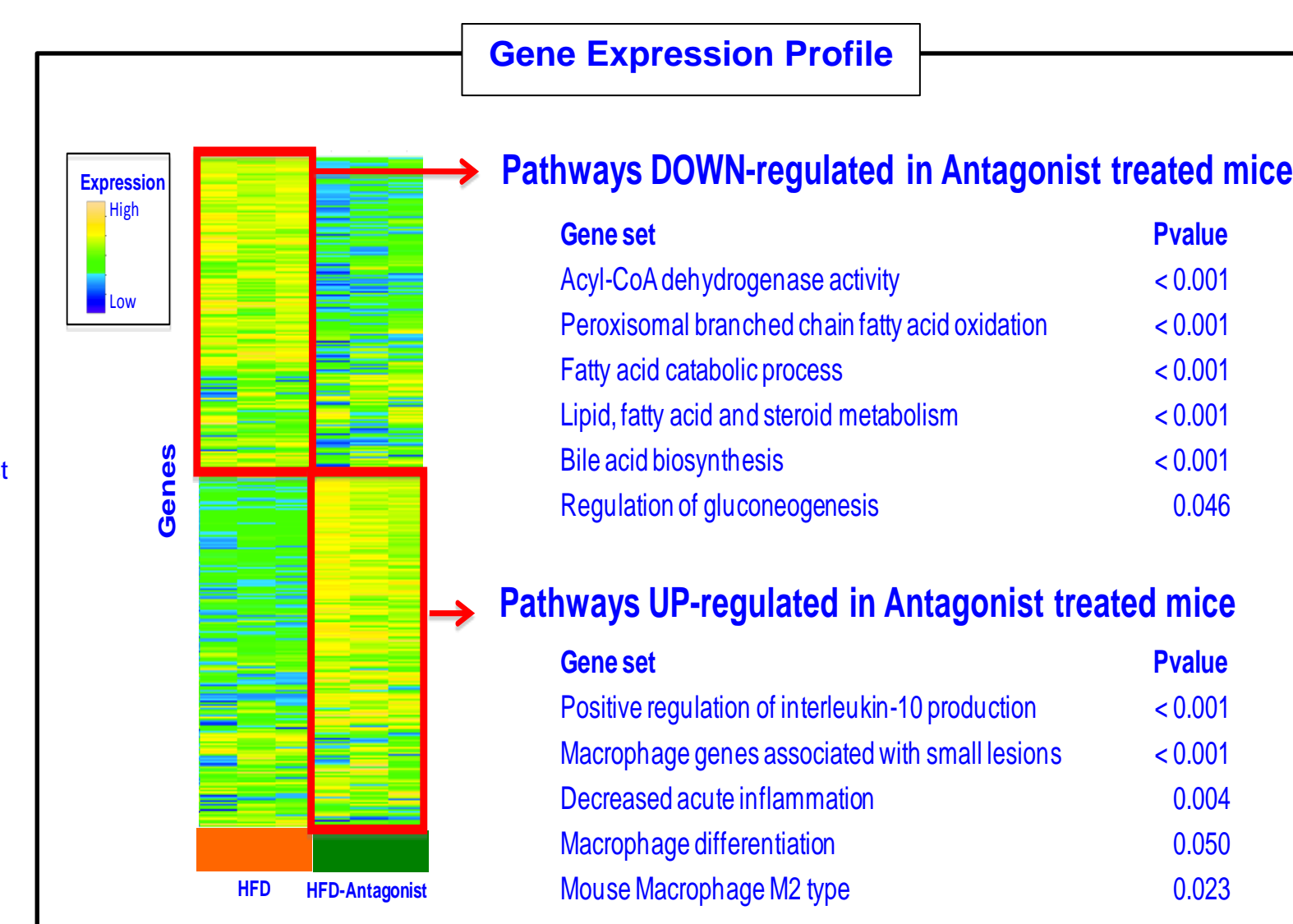
Antagonist Decreases Serum Leptin Levels



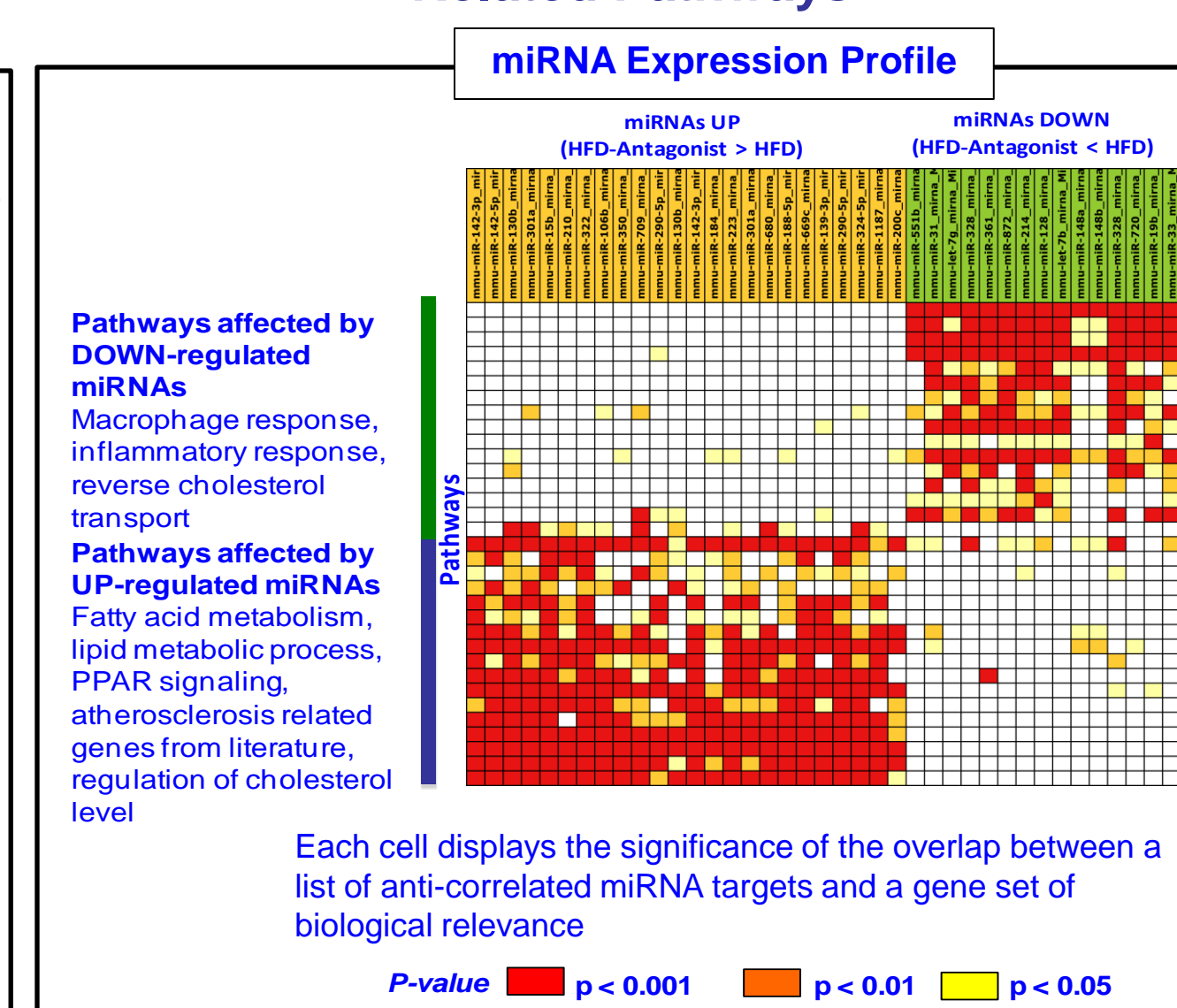
Antagonist Increases Fecal Cholesterol Excretion



Pathways Affected by Antagonist Treatment



miRNAs Perturbed by Antagonist Treatment Co-Target Cholesterol-Related Pathways



Real time PCR, Liver mRNA

	HFD-Antagonist (fold change)	HFD (fold change)
LXR	1.63 ± 0.18*	1.09 ± 0.08
PPARg	2.60 ± 0.24*	1.69 ± 0.48
IL10	36.70 ± 12.41*	1.22 ± 0.31
ABCG1	9.89 ± 2.53*	1.46 ± 0.44
ABCA1	1.12 ± 0.11	1.52 ± 0.41

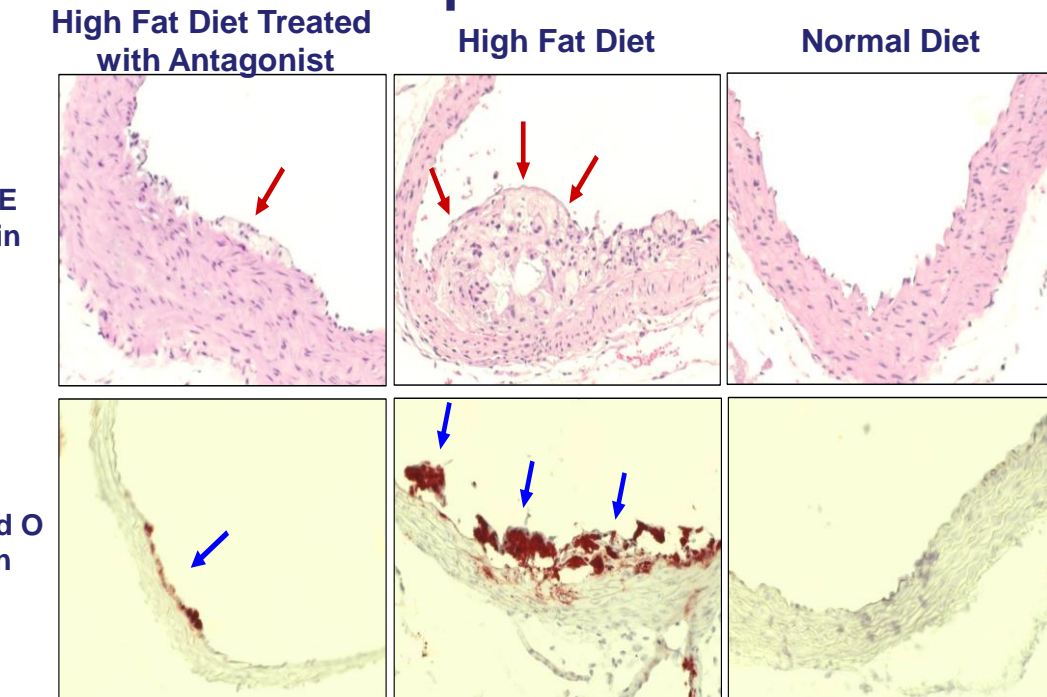
Real time PCR, Intestine mRNA

	HFD-Antagonist (fold change)	HFD (fold change)
LXR	-5.51 ± -3.71*	1.1 ± 0.09
PPARg	3.82 ± 0.34*	1.37 ± 0.46
IL10	55.22 ± 25.28*	1.18 ± 0.16
ABCG5	-3.22 ± -1.17*	1.12 ± 0.20
ABCG8	-3.80 ± -1.98*	1.09 ± 0.22
NPC1L1	-1.81 ± -0.14*	1.31 ± 0.31

*, P < 0.05 vs High Fat Diet group; N = 5

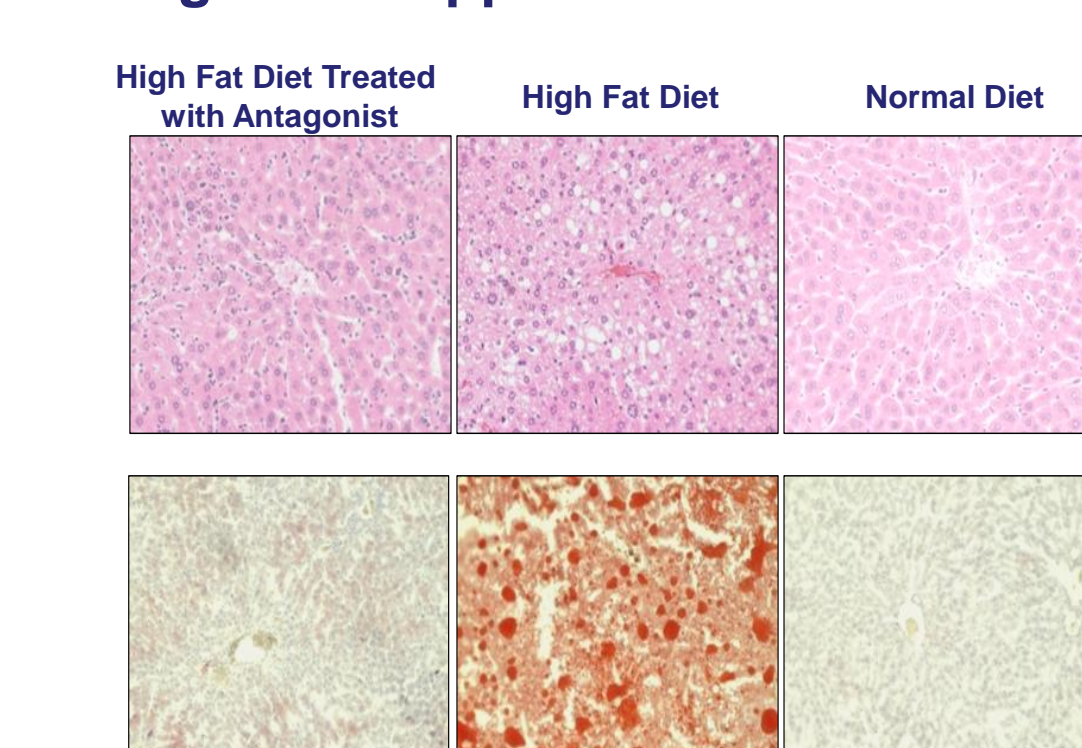
ApoE^{-/-}

Antagonist Suppresses HFD-induced Plaque Formation



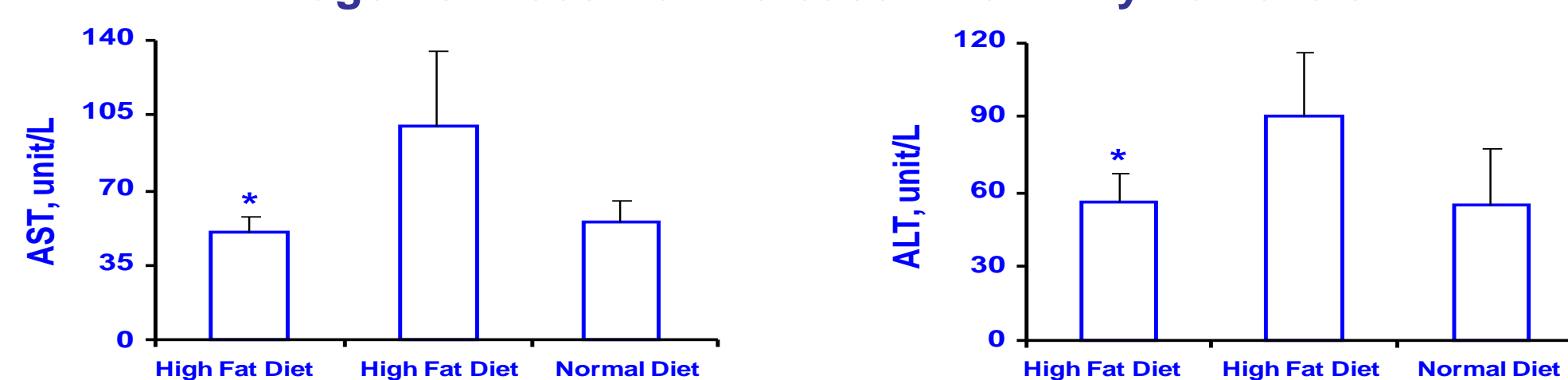
Arrows indicate atherosclerotic plaques at roots of aorta with many foam cells of macrophage origin (↑) and lipid contents (↑), 200x magnification. Data shown are from 15 mg/kg, 2 inj/week.

Antagonist Suppresses Liver Steatosis



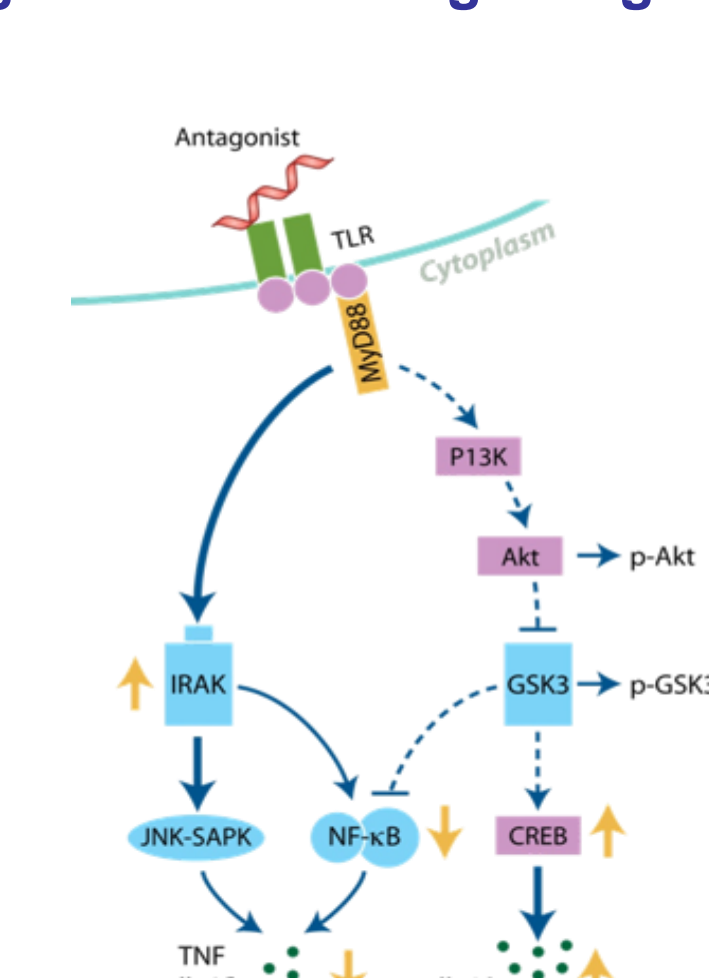
Lipid droplets are shown as oval empty spaces (H&E stain) or red color deposits (Oil red O stain) inside hepatocytes, 200x magnification. Data shown are from 15 mg/kg, 2inj/week.

Antagonist Does Not Increase Liver Enzyme Levels



*, P < 0.05 vs High Fat Diet group; not significant vs Normal Diet group; N = 15. Data shown are from 15 mg/kg, 2 inj/week

Working Hypothesis of the Effect of Antagonist on TLR Signaling Pathway



CONCLUSIONS

- TLR7 and TLR9 antagonist exerts therapeutic effects in mice fed on HFD
 - Reduces serum TC, LDL-c and liver triglycerides.
 - Reduces liver steatosis and plaque formation in arterial wall.
 - Reduces HFD-induced increase of liver enzymes and leptin.
 - TC lowering is inversely associated with antagonist-induced IL-10 levels.
 - Activation of PPAR_γ/LXR/ABCA1/ABCG1 pathway is linked to TC lowering and enhanced fecal excretion of cholesterol.
- Data suggest involvement of TLR7 and TLR9 in diet-induced hyperlipidemia and atherosclerosis.
- Observation is in agreement with high incidence of atherosclerosis and CVD in SLE and RA where the activation of TLRs is thought to contribute to chronic inflammation and disease development.

Authors Disclosures: FZ, WJ, MJR, DY, ERK, NLM and SA are employees of Idera Pharmaceuticals, Inc. PU and WM have no disclosures