Supplemental Methods

Cholesterol efflux study with bone marrow macrophages

Bone marrow macrophages (BMMs) were isolated from femurs and tibias and cultured in Dulbecco modification of Eagle media (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 30 % L929 conditioned medium for 7 days[1]. BMMs were grown in 12-well Petri dishes, labeled with 5 μCi/ml [3H]-cholesterol, and loaded with or without 25 μg/ml acetylated LDL (acLDL). Twenty-four hours later, cells were washed and equilibrated overnight in DMEM-0.2% bovine serum albumin (BSA) with or without 10 μM GW3965. The cells were washed with PBS and cholesterol efflux was initiated by the addition of DMEM-0.2 % BSA with or without 25 μg/mL HDL3. After 4 hours incubation, aliquots of the medium were filtered by MultiScreen-HV filter plates from Millipore to remove the floating cells and the supernatants were counted in a liquid scintillation counter (LSC). The cells were extracted with 2-propanol, evaporated under nitrogen gas, resuspended in toluene, and counted in an LSC. Cholesterol efflux was expressed as percentage of total tritium radioactivity presented in the cells plus the effluxed medium.

Hepatic Triglyceride and Cholesterol Measurements

Livers were perfused and homogenized in saline using 3 ml/g liver. The homogenates were diluted 5X with PBS and lipids solubilized at 37 degrees for 5 minutes in 1 % deoxycholate for triglyceride or 0.25 % deoxycholate for cholesterol. Triglyceride and cholesterol levels were measured with the use of diagnostic reagents from Thermo Scientific.

Reference

1. Schiller NK, Black AS, Bradshaw GP, Bonnet DJ, Curtiss LK. Participation of macrophages in atherosclerotic lesion morphology in LDLr-/- mice. *J Lipid Res.* 2004;45:1398-1409.

Supplemental legends

Supplemental Figure I. Structure of systemic LXR agonist GW3965 and intestine-specific LXR agonist GW6340.

Supplemental Figure II. BMMs from wild-type (WT) or LXR DKO mice were labeled with [3H]-cholesterol and loaded with or without 25 μg/ml acetylated LDL (acLDL) for 24 hours. Cells were equilibrated for 18 hours in the presence or absence of 1 μM (GW3965). Cholesterol efflux was determined in the absence (A) or presence of HDL3(25 μg/ml) (B) for 4 hours. Data are expressed as mean ± SD; n = 3. $^{**}P < 0.01$.

Table I . Effects of 5-day GW3965 treatment on plasma lipid levels

Mice	Drug	Total cholesterol	HDL cholesterol	Triglyceride
Wild type	**Vehicle**	96.8 ± 9.6	73 ± 9.7	61.7 ± 18.9
	GW3965	142.8 ± 14.6 **	110.8 ± 12.2 **	68.7 ± 13.6
LXR KO	**Vehicle**	102.2 ± 12.5	68.8 ± 11	33.3 ± 9.9 §§
	GW3965	98.5 ± 15.1	65.3 ± 23.6	33 ± 7.4 §§

Values are means ± SD in mg per 100 mL plasma. In each group, n=6 samples were determined. See Methods for details on diets and lipid analysis. **p<0.01 vs vehicle group. §§p<0.01 vs wild type mice.

Table II . Effects of 12-day GW3965 and GW6340 treatment on hepatic lipid levels

Mouse	Drug	Cholesterol	Triglyceride
Wild type	**Vehicle**	3.11 ± 0.42	4.3 ± 0.5
Wild type	**GW6340**	3.18 ± 0.72	4.0 ± 1.34
Wild type	**GW3965**	2.91 ± 0.33	7.14 ± 1.03 **

Values are means ± SD in mg per g liver. In each group, n=5 samples were determined. See Methods for details on lipid analysis. **p<0.01 vs vehicle and GW6340 group.

Table III . Effects of 5-day GW3965 and GW6340 treatment on plasma lipid levels

Mice	Drug	Total cholesterol	HDL cholesterol	Triglyceride
Wild type	**Vehicle**	89 ± 4.3	56.8 ± 7.4	44 ± 7.4
Wild type	**GW6340**	107.9 ± 23.5	62.5 ± 10.5	47.5 ± 16
Wild type	**GW3965**	131.5 ± 15.7**	100.3 ± 15.9**§§	51.3 ± 10.4

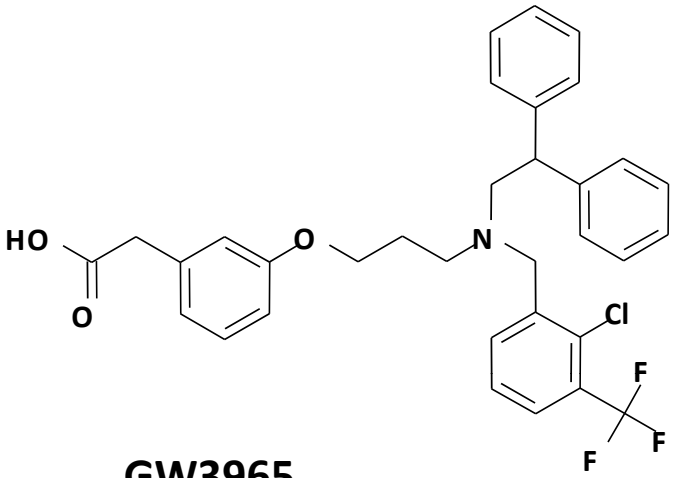
Values are means ± SD in mg per 100 mL plasma. In each group, n=5 samples were determined. See Methods for details on diets and lipid analysis. **p<0.01 vs vehicle group. §§p<0.01 vs GW6340 group

Table IV . Effects of 5-day GW3965 treatment on plasma lipid levels

BMM	Drug	Total cholesterol	HDL cholesterol	Triglyceride
WT	Vehicle	82.3 ± 4.6	67 ± 5.0	44.5 ± 11.2
WT	GW3965	132.2 ± 9.4**	107.7 ± 8.6**	49.7 ± 12.7
LXR DKO	Vehicle	87.5 ± 6.8	71 ± 7.1	42.2 ± 14.2
LXR DKO	GW3965	130.0 ± 6.7**	105.2 ± 5.2**	46.5 ± 8.4

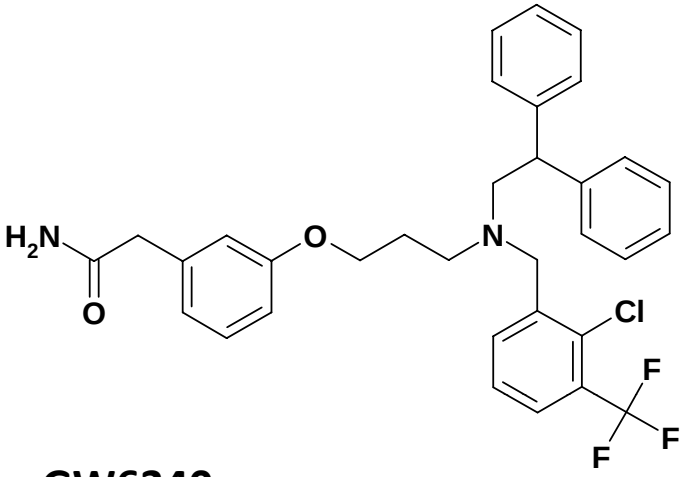
Values are means ± SD in mg per 100 mL plasma. In each group, n=6 samples were determined. See Methods for details on diets and lipid analysis. WT and LXR DKO indicates BMMs from wild-type mice and LXR DKO mice, respectively. **p<0.01 vs vehicle group.

Supplemental Figure.I



GW3965

LXR transactivation pEC50=6.7



GW6340

LXR transactivation pEC50=7

Supplemental Figure II