

Inhibition of cholesterol absorption associated with a PPAR α -dependent increase in ABC binding cassette transporter A1 in mice

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Abstract Dietary supplementation with the peroxisome proliferator-activated receptor α (PPAR α) ligand WY 14,643 gave rise to a 4- to 5-fold increase in the expression of mRNA for the ATP binding cassette transporter A1 (ABCA1) in the intestine of normal mice. There was no effect in the intestine of PPAR α -null mice. Consumption of a high-cholesterol diet also increased intestinal ABCA1 expression. The effects of WY 14,643 and the high-cholesterol diet were not additive. WY 14,643 feeding reduced intestinal absorption of cholesterol in the normal mice, irrespective of the dietary cholesterol concentration, and this resulted in lower diet-derived cholesterol and cholesteryl ester concentrations in plasma and liver. At each concentration of dietary cholesterol, there was a similar significant inverse correlation between intestinal ABCA1 mRNA content and the amount of cholesterol absorbed. The fibrate-induced changes in the intestines of the normal mice were accompanied by an increased concentration of the mRNA encoding the sterol-regulatory element binding protein-1c gene (SREBP-1c), a known target gene for the oxysterol receptor liver X receptor α (LXR α). There was a correlation between intestinal ABCA1 mRNA and SREBP-1c mRNA contents, but not between SREBP-1c mRNA content and cholesterol absorption. **Key words:** These results suggest that PPAR α influences cholesterol absorption through modulating ABCA1 activity in the intestine by a mechanism involving LXR α .—Knight, B. L., D. D. Patel, S. M. Humphreys, D. Wiggins, and G. F. Gibbons. **Inhibition of cholesterol absorption associated with a PPAR α -dependent increase in ABC binding cassette transporter A1 in mice.** *J. Lipid Res.* 2003. 44: 2049–2058.

Supplementary key words intestine • sterol-regulatory element binding protein • liver X receptor • peroxisome proliferator-activated receptor- α null mice • ω -3 unsaturated fatty acid

The ATP binding cassette transporter A1 (ABCA1) is a membrane-bound protein that is thought to facilitate the

transport of cholesterol and phospholipids out of cells (1). Deficiency of ABCA1 gives rise to Tangier disease and familial HDL deficiency, both of which are characterized by a low concentration of HDL in plasma (2–5). In Tangier disease, cholesteryl esters accumulate in macrophage-derived foam cells present in tonsils, liver, and intestinal mucosa (6). Consistent with this observation, it has been shown that primary and transformed macrophages increase ABCA1 mRNA content and cholesterol efflux in response to cholesterol loading and oxysterols (5, 7–9). Thus, ABCA1 could be an important component of the apolipoprotein A1-mediated reverse cholesterol transport pathway for the removal of cholesterol from macrophages and other peripheral cells and its return to the liver as HDL.

Further evidence using ABCA1-null mice suggests that ABCA1 may have an additional role in maintaining whole-body cholesterol homeostasis by transporting dietary cholesterol out of intestinal absorptive cells and returning it to the intestinal lumen (10). However, this conclusion is somewhat controversial, because a second study has reported that ABCA1 deficiency gave rise to a decrease in cholesterol absorption (11). Nevertheless, in normal mice, administration of a synthetic ligand for retinoid X receptor (RXR) markedly stimulated intestinal ABCA1 mRNA content while abolishing net absorption of dietary cholesterol (8). The heterodimeric partner for RXR in regulating ABCA1 transcription in the gut was liver X receptor (LXR), which had previously been shown to mediate the effects of oxysterols on ABCA1 mRNA levels in macrophages (7, 12).

A second partner of RXR, peroxisome proliferator-activated receptor (PPAR), has also been implicated in the control of expression of ABCA1 in macrophage foam cells (13), although the mechanism remains obscure and it is

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not known if it operates in other cell types. PPARs regulate a number of important genes involved in lipid and glucose metabolism and are activated by unsaturated fatty acids and their derivatives (14). The fibrate group of drugs, used extensively as lipid-lowering agents, are pharmacological activators of PPAR α , the form found mainly in liver, muscle, and intestine (15). A role for PPAR α in modulating intestinal cholesterol metabolism was suggested by the observation that gemfibrozil, a pharmacological ligand of PPAR α , suppressed cholesterol absorption from the gut into mesenteric lymph in rats (16). Elucidation of the physiological role of PPAR α in the control of lipid metabolism has been simplified by the availability of a PPAR α knockout mouse in which the ligand binding domain has been disrupted (17). These animals have an increased plasma HDL concentration (18) and reduced mitochondrial β -oxidation (19), with an impaired response to fasting (20, 21) and a disturbed circadian rhythm of genes regulating fatty acid and cholesterol synthesis (22). In this report, we have used normal and PPAR α -null mice to examine whether PPAR α is involved in the regulation of cholesterol absorption through changes in intestinal ABCA1.

MATERIALS AND METHODS

PPAR α -null mice (17) bred onto a SV/129 genetic background were kindly provided by Dr. J. Peters and Dr. F. J. Gonzalez (National Institutes of Health, Bethesda, MD). Wild-type SV/129 mice were used as controls. All mice were male, and were used between the ages of 14 and 20 weeks. Animals were maintained in temperature-controlled rooms (22–24°C) on a 12 h light/12 h dark cycle (22). When required, the commercial high-carbohydrate pelleted diet containing 0.02% cholesterol (Special Diet Service, Witham, Essex, UK) was supplemented with 2% cholesterol (>99% purity; Sigma-Aldrich, Dorset, UK) or 0.1% WY 14,643 (ChemSyn Science Laboratories, Lenexa, KS) and fed for 7 days. Food, and thus cholesterol consumption, was measured on a daily basis, and was similar for each genotype. Mice were anaesthetized with pentobarbital, and samples of blood were taken from the descending vena cava. The proximal intes-

tine between approximately 5 and 15 cm from the stomach was removed, rinsed with ice-cold Dulbecco's phosphate-buffered saline, and frozen in liquid nitrogen. All procedures were conducted with the appropriate ethical approval.

The frozen tissues were ground to a powder under liquid nitrogen, and total RNA was extracted from portions of powder using the RNazol kit (Biogenesis, Poole, UK). The amount of mRNA was assayed by reverse transcription (23) followed by real-time PCR using the ABI PRISM Sequence Detection System (PE Applied Biosystems, Foster City, CA) using the primers and probes as given previously (22) or as shown in **Table 1**. Reactions were carried out in triplicate in 30 μ l of TaqMan Universal PCR Master Mix with β -actin as internal standard (22). All values were related to a curve generated by a standard liver or intestinal preparation and were corrected for β -actin mRNA content. ABCA1 protein was detected by immunoblotting the intestinal membrane fraction (24) using a specific antipeptide polyclonal antibody raised in rabbits to residues 1,201–1,211 of the human ABCA1 protein (ab7360, from Abcam, Cambridge, UK). Blots were scanned to give a semiquantitative estimate of changes in intensity.

A previously described method using the nonabsorbed plant sterol marker sitostanol (25) was adapted to measure cholesterol absorption in the present work. Trace quantities of [4-¹⁴C]cholesterol and [5,6-³H]sitostanol were incorporated into the appropriate diet (\sim 20 μ Ci and \sim 25 μ Ci, respectively, per 100 g chow) and fed for the final 2 days of the 7 day regimen. Portions of the different types of diet were used for measurement of the exact ³H/¹⁴C sterol ratios following lipid extraction. For the chow, chow + WY 14,643, high-cholesterol, and high-cholesterol + WY 14,643 diets, these values were 0.84, 0.82, 0.74, and 0.75, respectively. The feces were collected after the final 2 day period, frozen in liquid nitrogen, ground to a powder, and an aliquot used for total lipid extraction (26). A part of the extract was used to determine the ³H/¹⁴C ratio by scintillation counting. The remainder was subjected to thin-layer chromatography (27) to separate the esterified and nonesterified sterol fractions.

Plasma and tissue lipid concentrations were assayed as described previously (22) following solvent extraction. Labeled esterified and nonesterified sterols were separated by thin-layer chromatography of total lipid extract (27) and their radioactivities assayed by scintillation counting using a Beckman LS 6500 scintillation counter. Tissue protein content was determined by the method of Lowry et al. (28). Statistical analysis was carried out using Student's *t*-test for unpaired data.

TABLE 1. Sequences of oligonucleotides used as primers and labeled probes for real-time PCR (TaqMan) assays

cDNA	Primer or Probe	Oligonucleotide Sequences
ABCA1	Forward	5'-TTGGCGCTCAACTTTTACGAA-3'
	Probe	5'-FAM-CCCAACTTCTGGCAGCGCCTACATC-TAMRA-3'
	Reverse	5'-GAGCGAATGTCCTTCCCCA-3'
SREBP-2	Forward	5'-CAAGTCTGGCGTCTGAGGAA-3'
	Probe	5'-FAM-CCATTGATTACATCAATATCTGCAGCAGGTCAA-TAMRA-3'
	Reverse	5'-ATGTTCTCCTGGCGCAGCT-3'
SREBP-1a	Forward	5'-AGGCGGCTCTGGAACAGA-3'
	Probe	5'-FAM-TGGCCGAGATGTGCCAACTGGA-TAMRA-3'
	Reverse	5'-ATGTCGTTCAAACCGCTGTGT-3'
SREBP-1c	Forward	5'-ATCGGCGCGGAAGCTGTCGGGGTAGCGTC-3'
	Probe	5'-FAM-CGGAGCCATGGATTGCACATTTGA-TAMRA-3'
	Reverse	5'-ACTGTCTTGGTTGTTGATGAGCTGGAGCAT-3'
LXR α	Forward	5'-CTGCACGCCTACGTCTCCAT-3'
	Probe	5'-FAM-ACCACCCCAACGACCGACTGATG-TAMRA-3'
	Reverse	5'-AAGTACGGAGGCTCACAGCTT-3'

ABCA1, ATP binding cassette transporter A1; LXR α , liver X receptor α ; SREBP, sterol-regulatory element binding protein.

Intestinal ABCA1 and cholesterol absorption

Wild-type and PPAR α -null mice were fed for 7 days with diets containing high and low concentrations of cholesterol, either alone or in combination with the potent fibrate, WY 14,643. Changes in the abundance of intestinal ABCA1 mRNA were then compared with changes in cholesterol absorption from the gut. In these experiments, all animals were killed during the light phase of the diurnal cycle, when stimulation of ABCA1 mRNA expression by the PPAR α ligand was greatest. The animals were apparently unaffected by the dietary supplements, as evidenced by a similarity in food intake and overall weight. The high-cholesterol diet produced a slight increase in hepatic triacylglycerol content in the normal animals ($29.6 \pm 6.1 \mu\text{mol/g}$ to $41.3 \pm 4.3 \mu\text{mol/g}$; $P < 0.05$), but not of the already raised level in the PPAR α -null animals ($50.5 \pm 3.0 \mu\text{mol/g}$ to $58.7 \pm 12.8 \mu\text{mol/g}$). No other evidence of steatosis was observed.

Feeding the high-cholesterol diet alone increased the intestinal expression of ABCA1 mRNA to the same extent in both the normal and the PPAR α -null mice (Fig. 1A). In-

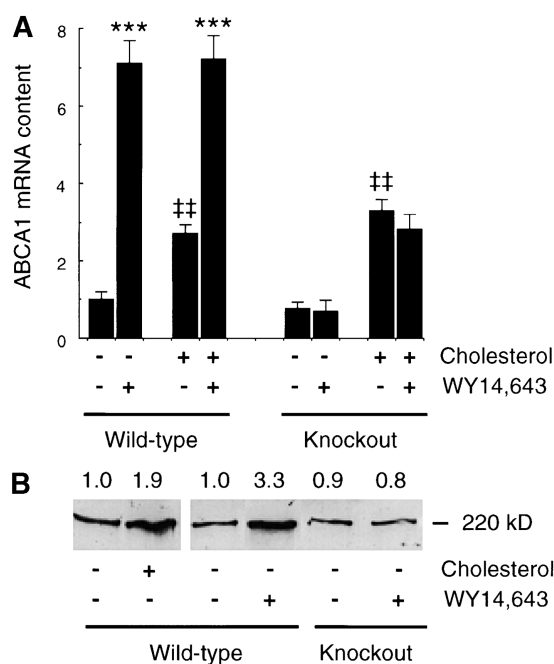


Fig. 1. The effects of fibrate and a high-cholesterol diet on intestinal ATP binding cassette transporter A1 (ABCA1) expression. Peroxisome proliferator-activated receptor α (PPAR α)-null (knockout) and wild-type mice were fed a diet with (+) or without (-) 0.1% WY 14,643, or a high-cholesterol (2%) diet for 7 days, and were killed at the midpoint of the light phase of the cycle. **A:** Intestinal ABCA1 mRNA was assayed and the tissue content expressed relative to a standard preparation. Results are shown as mean \pm SEM for four animals in each group. Values marked *** showed significant ($P < 0.001$) effects of WY 14,643, and ## indicates a significant ($P < 0.01$) effect of cholesterol. **B:** Intestinal membranes from four mice were pooled and subjected to Western blotting, developed with a polyclonal antibody against ABCA1. Values above the blots are the fold change from the wild type on the standard diet.

clusion of WY 14,643 in the low-cholesterol diet increased ABCA1 mRNA expression several-fold in the wild-type animals, but there was no response in the knockouts. Immunoblotting confirmed that these changes in mRNA level gave rise to similar changes in ABCA1 protein (Fig. 1B). In normal mice, inclusion of WY 14,643 in the high-cholesterol diet further increased the expression of intestinal ABCA1 mRNA, but only to a value similar to that with WY 14,643 alone (Fig. 1A). In PPAR α -null mice, inclusion of WY 14,643 in the high-cholesterol diet did not further increase intestinal ABCA1 mRNA expression (Fig. 1A).

To measure cholesterol absorption, both the high- and low-cholesterol diets were labeled with trace amounts of [^{14}C]cholesterol and [^3H]sitostanol. Because sitostanol is not absorbed from the intestine and is excreted quantitatively with the feces, comparison of the $^3\text{H}/^{14}\text{C}$ ratio of the fecal nonesterified sterols with that of the dietary sterols provided a measure of the proportion of the dietary cholesterol that was absorbed (25). Addition of WY 14,643 to both the low-cholesterol and high-cholesterol diets significantly reduced the proportion of dietary cholesterol absorbed by the wild-type mice but had no effect on the PPAR α -null mice (Table 2). When expressed for each individual mouse, there was a significant inverse correlation between cholesterol absorption and the intestinal ABCA1 mRNA content at both high and low dietary cholesterol concentrations (Fig. 2A). A given increase in ABCA1 mRNA content was associated with a similar fall in absorption, irrespective of the amount of cholesterol in the diet (Fig. 2B).

Cholesterol concentrations in liver and plasma

To confirm the observed changes in cholesterol absorption, the amount of total and dietary-derived [^{14}C]cholesterol was determined in both liver and blood plasma. Under no conditions could we detect any tritium in the plasma, confirming that none of the dietary [^3H]sitostanol had been absorbed. Thin-layer chromatography of the extracted lipids showed that in each case, the [^{14}C] label was associated almost entirely with esterified and non-esterified cholesterol.

Changes in dietary [^{14}C]cholesterol absorption were reflected by parallel changes in the concentration of [^{14}C]labeled sterols in the liver. Feeding WY 14,643 to the normal mice, but not the PPAR α -null mice, was accompanied by a 50% decrease in the amount of hepatic free [^{14}C]cholesterol and a 65% decrease in [^{14}C]cholesteryl ester (Table 3). Feeding cholesterol increased the proportion of [^{14}C]cholesterol that became esterified. Inclusion of WY 14,643 in the high-cholesterol diet decreased the amount of diet-derived free and, particularly, esterified [^{14}C]cholesterol in the livers of normal mice, so that there was a significant reduction in the proportion that was esterified. These changes in supply of dietary cholesterol in the normal mice gave rise to significant changes in the total hepatic cholesterol content (Table 3). Cholesterol feeding doubled the amount of cholesterol in the liver, and inclusion of fibrate in the low- and high-cholesterol diets re-

TABLE 2. Effects of WY 14,643 on absorption of dietary cholesterol

Genotype	Dietary Cholesterol	WY 14,643	Cholesterol Absorption (Absorbed/Ingested)	Total Cholesterol Absorbed
				mg/day
Wild type	Low	–	0.690 ± 0.033	0.51 ± 0.02
	Low	+	0.568 ± 0.048 ^a	0.42 ± 0.03 ^a
	High	–	0.124 ± 0.014	11.36 ± 1.31
	High	+	0.096 ± 0.006 ^a	7.83 ± 0.50 ^a
PPAR α -null	Low	–	0.723 ± 0.031	0.56 ± 0.02
	Low	+	0.759 ± 0.039 ^b	0.59 ± 0.03 ^c
	High	–	0.138 ± 0.010	11.82 ± 0.88
	High	+	0.143 ± 0.011 ^c	12.01 ± 0.91 ^c

PPAR, peroxisome proliferator-activated receptor. Normal and PPAR α -null mice were fed a low- (0.02% w/w) or high- (2.0% w/w) cholesterol diet supplemented (+) or not supplemented (–) with WY 14,643 (0.1% w/w) for 7 days. Values are expressed as the mean (n = 4 in each group) \pm SEM.

^aSignificantly different ($P < 0.05$) from those obtained from mice fed the corresponding diet without WY 14,643.

^bSignificantly different ($P < 0.05$) from the wild-type animals fed the corresponding diet.

^cSignificantly different ($P < 0.01$) from the wild-type animals fed the corresponding diet.

duced it by 30% and 50%, respectively. The hepatic cholesterol content of the PPAR α -null mice was higher than that of the wild type on the low-cholesterol diet, and was not affected by WY 14,643. The proportion of the liver cholesterol derived from the diet was about 5% for animals on the low-cholesterol diet and 80% for those on the high-cholesterol diet (Table 3). These proportions were reduced by fibrate in the wild-type but not in the PPAR α -null animals.

Blood plasma showed changes similar to although less pronounced than those in liver, with significant reductions in [¹⁴C]cholesterol content after feeding WY 14,643 (Table 4). Cholesteryl ester content was particularly resistant to change, although small significant effects of fibrate were observed in the normal animals on the high-cholesterol diet. In the absence of cholesterol or fibrate, the PPAR α -null animals retained significantly more of the dietary-derived cholesterol in the plasma than did the wild type (Table 4).

The content of total [¹⁴C]cholesterol in the intestinal wall showed no significant difference between PPAR α -null mice and normals, on either the low-cholesterol diet (619 ± 147 dpm $\times 10^{-3}$ /g vs. 647 ± 191 dpm $\times 10^{-3}$ /g) or the high-cholesterol diet (72 ± 13 dpm $\times 10^{-3}$ /g vs. 110 ± 20 dpm $\times 10^{-3}$ /g), and was not significantly increased by WY 14,643 in the normal mice (values of 691 ± 110 dpm $\times 10^{-3}$ /g and 80 ± 12 dpm $\times 10^{-3}$ /g on low- and high-cholesterol diets, respectively).

Involvement of intestinal LXR α

If PPAR α acts via LXR α in the intestine, as it does in macrophages (13), it might be expected that activation by WY 14,643 would enhance the mRNA concentration of other LXR α -responsive genes, such as sterol-regulatory element binding protein-1c (SREBP-1c) (29). To examine this, we assayed the intestinal mRNA content of SREBP-1c and compared it to that of the other SREBP isoforms. Inclusion of WY 14,643 in the diet produced a small increase in the mRNA content of SREBP-1a and SREBP-2, but had a much larger effect on SREBP-1c (Fig. 3). This response to

the drug was absent in the intestines of the PPAR α -null mice. The patterns of SREBP mRNA expression were reflected by changes in the mRNA content of their own target genes, fatty acid synthase and acetyl-CoA carboxylase for SREBP-1c, and HMG-CoA reductase for SREBP-2 (Fig. 3).

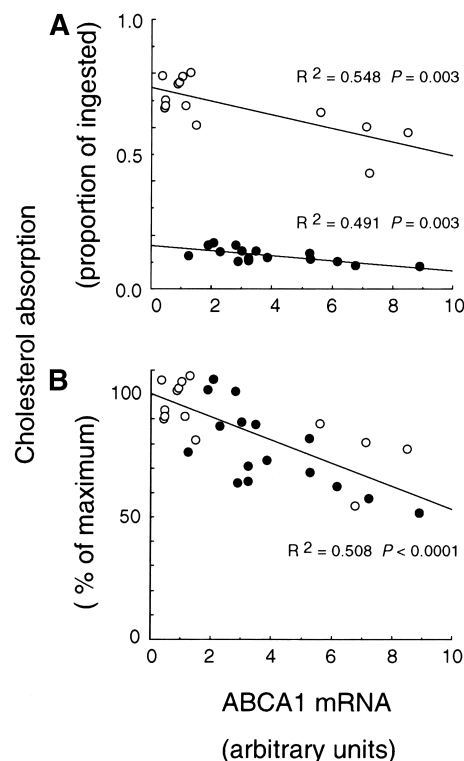


Fig. 2. Relationship between intestinal ABCA1 mRNA content and cholesterol absorption. A: Total ABCA1 mRNA content was compared with the proportion of the dietary cholesterol that was absorbed. Each point represents values from an individual mouse fed the low-cholesterol (0.02%) (open circles) or the high-cholesterol (2%) (closed circles) diet. B: Values for absorption were expressed as a percentage of the maximum for each diet, determined from the intercepts on the y axis in panel A.

TABLE 3. Effects of WY 14,643 on diet-derived and total cholesterol in the liver

Genotype	Dietary Cholesterol	WY 14,643	[¹⁴ C] in Liver Cholesterol			Liver Cholesterol		
			Free	Esterified	Proportion of [¹⁴ C]cholesterol as Ester	Total	Proportion as Ester	Dietary-Derived Cholesterol
			<i>dpm</i> × 10 ⁻³ /g	<i>dpm</i> × 10 ⁻³ /g	%	μmol/g	%	μmol/g
Wild type	Low	-	449.3 ± 47.1	122.2 ± 6.1	21.7 ± 1.3	12.23 ± 0.97	11.4 ± 2.5	0.538 ± 0.049
	Low	+	241.4 ± 45.8 ^a	45.8 ± 6.0 ^c	16.9 ± 3.2	8.51 ± 0.41 ^a	17.6 ± 9.9	0.271 ± 0.044 ^b
	High	-	80.8 ± 4.9	112.9 ± 9.5	58.1 ± 2.9	22.32 ± 1.77	35.4 ± 4.1	18.267 ± 0.866
PPARα-null	High	+	39.3 ± 2.1 ^c	17.9 ± 3.2 ^c	30.7 ± 3.7 ^b	10.99 ± 0.85 ^b	30.4 ± 5.7	5.401 ± 0.420 ^c
	Low	-	605.6 ± 94.6	194.5 ± 23.2 ^d	22.1 ± 3.2	17.24 ± 0.41 ^e	17.6 ± 9.2	0.765 ± 0.112
	Low	+	587.5 ± 40.5 ^d	144.3 ± 16.4 ^e	19.7 ± 2.0	15.07 ± 0.91 ^f	15.5 ± 12.1	0.690 ± 0.045 ^e
	High	-	85.2 ± 3.5	118.9 ± 13.7	57.7 ± 3.4	24.22 ± 4.26	45.8 ± 13.5	19.242 ± 1.222
	High	+	85.1 ± 7.1 ^f	136.4 ± 12.5 ^f	61.4 ± 2.9 ^f	28.39 ± 5.32 ^d	44.5 ± 22.3	20.876 ± 1.348 ^f

For details of the feeding schedule, see the legend to Table 2. All values are expressed as the mean (n = 4 in each group) ± SEM.

^a Significantly different (*P* < 0.05) from those obtained in mice fed the corresponding diet without WY 14,643.

^b Significantly different (*P* < 0.01) from those obtained in mice fed the corresponding diet without WY 14,643.

^c Significantly different (*P* < 0.001) from those obtained in mice fed the corresponding diet without WY 14,643.

^d Significantly different (*P* < 0.05) from the wild-type animals fed the corresponding diet.

^e Significantly different (*P* < 0.01) from the wild-type animals fed the corresponding diet.

^f Significantly different (*P* < 0.001) from the wild-type animals fed the corresponding diet.

Feeding a high-cholesterol diet increased intestinal SREBP-1c mRNA content 3- to 4-fold in both wild-type and PPARα-null mice (Fig. 4B). It had no effect on the mRNA levels for SREBP-1a (mean values ± SEM, for wild-type mice, of 0.863 ± 0.102 arbitrary units, without and 1.014 ± 0.118 with cholesterol) or SREBP-2 (0.945 ± 0.091 without and 0.919 ± 0.196 with cholesterol). As for ABCA1, the effects of cholesterol and fibrate on SREBP-1c mRNA levels in wild-type animals were not additive (Fig. 4B). Over the whole range of diets and genotypes tested, there was a strong correlation between the intestinal content of SREBP-1c mRNA and that of ABCA1 (Fig. 5A). This suggests that the mechanism by which these compounds affect SREBP-1c may be the same as that by which they influence ABCA1. However, there was no indication that changes in SREBP-1c mRNA levels had any direct effect on cholesterol absorption. There was no significant

correlation, with either the high- or low-cholesterol diet, between SREBP-1c mRNA content and the proportion of dietary cholesterol absorbed (Fig. 5B).

Intestinal LXRα mRNA content was the same in the normal and the PPARα-deficient mice, and was not affected by dietary manipulation (Fig. 4A).

DISCUSSION

The experiments presented here establish the existence of a strong inverse relationship between the expression of intestinal ABCA1 mRNA and the transfer of dietary cholesterol from the intestinal lumen into the body (Fig. 2). This observation provides strong support for the concept of ABCA1 acting as an intestinal gatekeeper to prevent excessive entry of exogenous cholesterol (30). Additionally,

TABLE 4. Effects of WY 14,643 on diet-derived and total cholesterol in the plasma

Genotype	Dietary Cholesterol	WY 14,643	[¹⁴ C] Label in Plasma		Plasma Cholesterol		Dietary-Derived Cholesterol
			Total	Proportion of [¹⁴ C]cholesterol as Ester	Total	Proportion as Ester	
			<i>dpm</i> × 10 ⁻³ /ml	%	mmol/l	%	
Wild type	Low	-	115.3 ± 4.8	70.1 ± 1.7	2.78 ± 0.07	59.4 ± 1.6	0.088 ± 0.008
	Low	+	71.9 ± 14.8 ^a	70.0 ± 1.0	2.26 ± 0.23	54.6 ± 1.4	0.062 ± 0.028
	High	-	19.1 ± 1.3	73.4 ± 0.5	3.03 ± 0.18	62.0 ± 0.5	1.677 ± 0.243
PPARα-null	High	+	12.9 ± 1.0 ^b	67.2 ± 1.5 ^b	3.00 ± 0.11	53.2 ± 2.2 ^b	1.110 ± 0.158 ^a
	Low	-	174.6 ± 11.8 ^c	70.7 ± 1.8	3.27 ± 0.31	61.1 ± 2.4	0.146 ± 0.035 ^c
	Low	+	161.4 ± 14.3 ^d	70.7 ± 2.4	3.03 ± 0.09 ^c	59.7 ± 4.4	0.128 ± 0.045 ^c
	High	-	18.4 ± 2.7	74.0 ± 0.5	3.41 ± 0.34	61.2 ± 1.1	2.154 ± 0.864
	High	+	20.8 ± 0.6 ^e	73.8 ± 0.8 ^d	3.45 ± 0.06 ^c	63.7 ± 1.2 ^d	1.838 ± 0.169 ^d

For details of the feeding schedule, see the legend to Table 2. Values are expressed as the mean (n = 4 in each group) ± SEM.

^a Significantly different (*P* < 0.05) from those obtained in mice fed the corresponding diet without WY 14,643.

^b Significantly different (*P* < 0.01) from those obtained in mice fed the corresponding diet without WY 14,643.

^c Significantly different (*P* < 0.05) from the wild-type animals fed the corresponding diet.

^d Significantly different (*P* < 0.01) from the wild-type animals fed the corresponding diet.

^e Significantly different (*P* < 0.001) from the wild-type animals fed the corresponding diet.

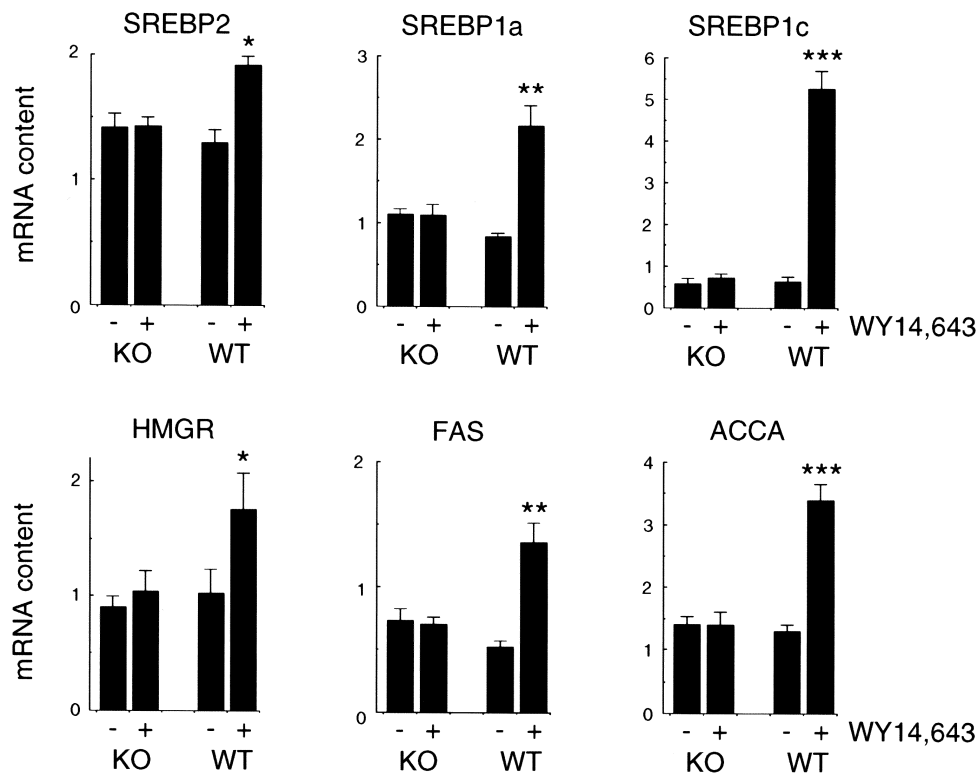


Fig. 3. The effect of fibrate on the intestinal mRNA content for sterol-regulatory element binding proteins (SREBPs), HMG-CoA reductase (HMGR), fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACCA). PPAR α -null (KO) and wild-type (WT) mice were fed a diet with (+) or without (-) 0.1% WY 14,643 for 7 days, and were killed at the midpoint of the light phase of the cycle. Results are shown as mean \pm SEM for between four and eight animals in each group. Values are given relative to a standard preparation and showed significant (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$) effects of WY 14,643.

it provides firm evidence that activation of intestinal PPAR α decreases dietary cholesterol absorption by a mechanism that involves an increase in intestinal ABCA1 mRNA and protein levels. This conclusion is supported by the antiparallel effects of the PPAR α ligand WY 14,643 on cholesterol absorption and ABCA1 mRNA levels in mice in which the PPAR α gene was intact, but a complete lack of either effect in mice in which the PPAR α gene was deleted. The physiological consequences of an inability to upregulate intestinal ABCA1 mRNA expression in a PPAR α -dependent manner include a continuing high rate of dietary cholesterol flux to the liver in PPAR α -null mice on a high-cholesterol diet, resulting in an almost 10-fold increase in hepatic cholesteryl ester compared with the wild type fed WY 14,643, and a significant increase in diet-derived and total cholesteryl ester in the blood plasma.

It is unlikely that PPAR α affects ABCA1 gene transcription directly, because the sequences of the ABCA1 promoter region that are conserved between human and mouse do not contain a typical PPAR α response element (31). However, the present results provide some clues as to the mechanism of the linkage between PPAR α activation and intestinal ABCA1 mRNA expression. It is known that activation of the oxysterol receptor LXR α in the intestine gives rise to an increase in ABCA1 expression and a reduction in cholesterol absorption (8). The most potent

natural LXR α ligands in vitro are monohydroxy-cholesterol derivatives with the hydroxyl group at position 22 or 24 on the side chain (32). The physiological importance of these ligands in vivo is yet to be established (33). However, it has been shown that the concentration of potential oxysterol ligands, in particular 24S-hydroxycholesterol, can be increased in the liver by increased dietary cholesterol (34). Thus, it seems probable that it was oxysterols derived from dietary cholesterol, acting through LXR α , that were responsible for the increased ABCA1 mRNA levels in the intestines of mice fed the high-cholesterol diet. The effects of cholesterol and WY 14,643 on ABCA1 mRNA levels in the intestine were not additive, suggesting that activation of PPAR α also increased ABCA1 mRNA by a mechanism that involved LXR α . To examine this possibility, we used SREBP-1c, a known LXR α target gene (29, 35, 36), as a reporter for LXR α -mediated responses. The results showed that activation of PPAR α by WY 14,643 increased expression of mRNA for SREBP-1c and its target genes in the intestines of the normal mice. This was not related to any general changes in cholesterol flux, because SREBP-2 mRNA levels were not similarly increased and were not affected by cholesterol feeding. WY 14,643 had no effect on SREBP-1c mRNA when the PPAR α gene was deleted, consistent with the idea that LXR α had not been activated in these animals. The closeness of the rela-

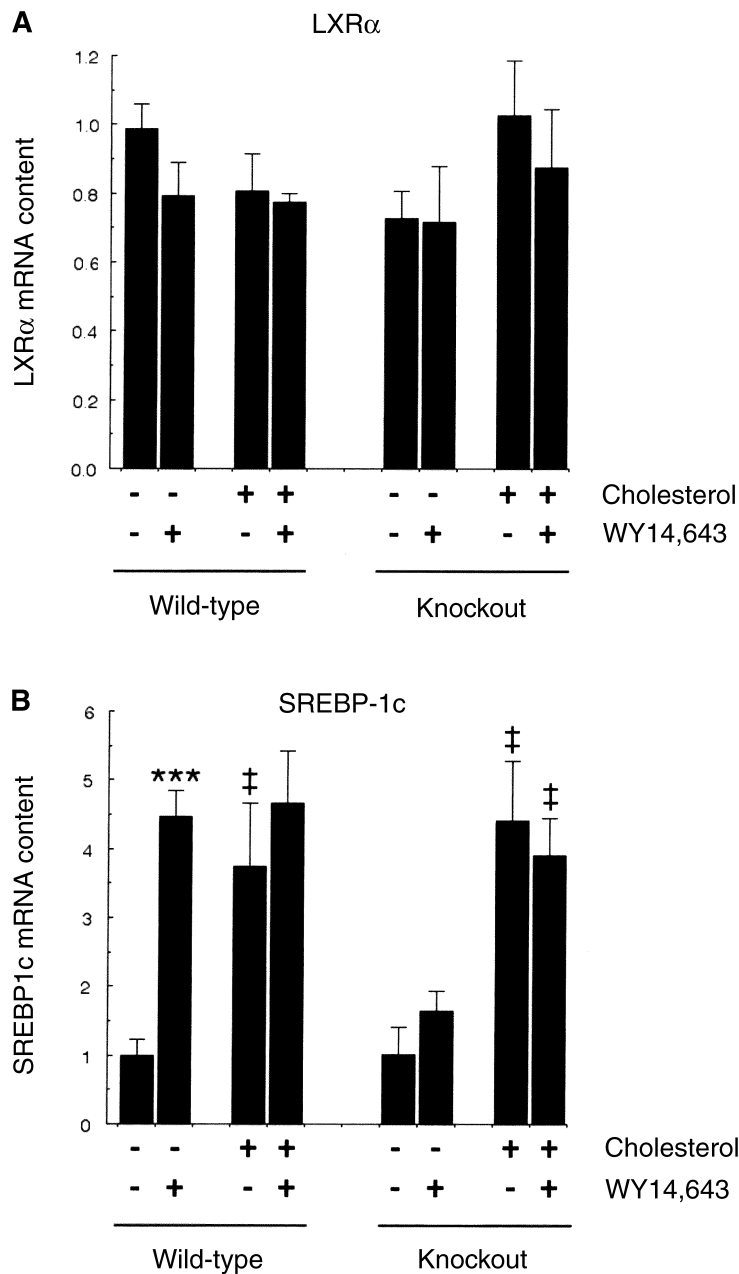


Fig. 4. The effects of fibrate and high cholesterol on intestinal liver X receptor α (LXR α) mRNA content (A) and SREBP-1c mRNA content (B). PPAR α -null and wild-type mice were treated as described in Fig. 1. LXR α and SREBP-1c mRNAs were assayed and the tissue content expressed relative to a standard preparation. Results are shown as mean \pm SEM for four animals in each group. Values marked *** showed significant ($P < 0.001$) effects of WY 14,643, and \ddagger indicates a significant ($P < 0.05$) effect of cholesterol.

tionship between the expression of ABCA1 and SREBP-1c mRNA also argues for the operation of a common mechanism. Despite this relationship, there was no correlation between SREBP-1c mRNA content and cholesterol absorption, indicating that SREBP-1c was probably not directly involved in any regulation of absorption by ABCA1.

In the intestine, we could find no evidence for a direct interaction between PPAR α and LXR α that inhibited transcription, as has been observed on promoter reporter constructs in vitro (37, 38), or for any apparent antagonism of LXR α activation (39) or function (40, 41) by PPAR α ligands, as observed in hepatoma cells. Furthermore, in contrast to the reported increase in LXR α mRNA in response to WY 14,643 and other fatty acid-derived PPAR α ligands in human macrophages (13) and liver cells (42) in

vitro, we could detect no change in LXR α mRNA expression in response to dietary WY 14,643 in the intestine. Thus, if LXR α is involved in transducing the PPAR α -mediated signal in the intestine, it must result from activation of existing LXR α . This conclusion is supported by the observation that cholesterol was unable to potentiate further the increase in expression of the ABCA1 or SREBP-1c genes after PPAR α activation by WY 14,643 in the intestines of the normal animals. Such a nonadditive response is characteristic of a mechanism that is saturated, consistent with the presence of limiting amounts of LXR α in the intestine.

Although the evidence suggests that cholesterol and fibrate ultimately influence ABCA1 expression through activation of LXR α , the activators need not be the same. In-

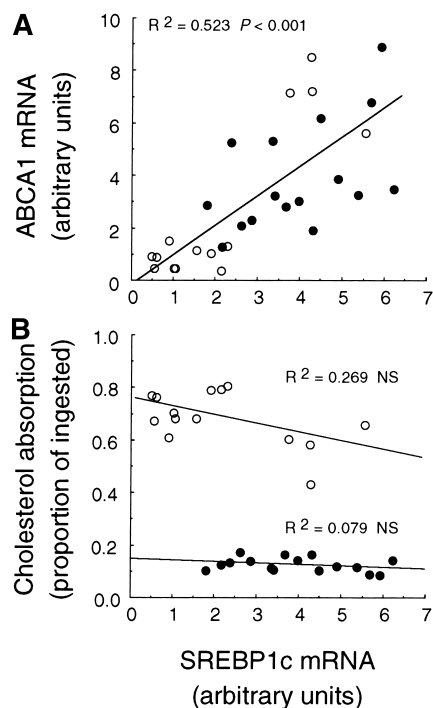


Fig. 5. Relationship between intestinal SREBP-1c mRNA content and (A) ABCA1 mRNA content or (B) cholesterol absorption. PPAR α -null and wild-type mice were treated as described in Fig. 1. Each point represents values from an individual mouse fed the low-cholesterol (0.02%) (open circles) or the high-cholesterol (2%) (closed circles) diet.

deed, the different origins of the responses would imply that they are not. The increase in ABCA1 mRNA content on cholesterol feeding is likely to be an adaptive response mediated by oxysterols derived from cholesterol in the diet. It occurs in PPAR α -deficient animals, and any increase in the return of cholesterol to the lumen does not prevent a net increase in cholesterol absorption. WY 14,643, on the other hand, is likely to be a causative agent, acting through a PPAR α -dependent pathway to increase ABCA1 expression and so affect absorption. The nature of the putative LXR α ligands produced by PPAR α activation is not known, although the evidence suggests that they could be particularly potent. Generally, the origins and mechanisms of formation of physiological oxysterols that could act as ligands for LXR α remain obscure, but members of the cytochrome P-450 superfamily are involved in the natural production of such compounds (43–45), and some, such as members of the CYP4A group, are known to be PPAR α target genes (14).

There was little, if any, difference in the basal levels of intestinal ABCA1 mRNA expression between the wild-type and PPAR α -null mice consuming the low-cholesterol diet alone. There was also no difference in intestinal cholesterol absorption. Therefore, differential cholesterol absorption cannot explain the net increase in diet-derived cholesterol and cholesteryl ester observed in the plasma and liver of the PPAR α -null mice compared with the normal mice fed the low-cholesterol diet (Tables 3 and 4). Al-

though PPAR α deficiency had little direct effect in intestine, it is known to lead to a number of changes in other tissues and in whole-body cholesterol metabolism that could explain the accumulation of cholesterol in these animals. These include a significantly decreased expression of ABCA1 mRNA in liver (data not shown), a redistribution of hepatic cholesterol between the esterified and nonesterified forms (22), and an impaired regulation of de novo cholesterol synthesis (22, 46).

In the present work, there was a decrease in plasma triacylglycerol concentration on feeding the wild-type mice with fibrate (from 646 ± 93 $\mu\text{mol/l}$ to 324 ± 37 $\mu\text{mol/l}$). It is known that PPAR α reduces CYP7A1 transcription in vitro (47) and that ciprofibrate decreases bile acid synthesis in rats, leading to a decline in fecal bile acid output (48). Thus, although it is probable that the decrease in triacylglycerol content mainly reflected an increase in hepatic fatty acid oxidation, it could, at least in part, have resulted from a decrease in absorption. On the basis of these results, a reduced intestinal bile acid content cannot be completely excluded as a factor contributing to the observed decrease in cholesterol absorption.

LXR α activation has no effect on intestinal ACAT and microsomal triglyceride transfer protein expression (49), suggesting that there was little change in the enzymes and other proteins involved in chylomicron assembly. Expression of ABCG5/G8 can also be regulated by LXR α . (50), although, because the exact specificity of this plant sterol transporter is not known, it is not possible to predict the contribution that it could make to the effects presented here. Likewise, we cannot rule out a role for the unknown targets of the cholesterol absorption inhibitor ezetimibe, which has recently been shown not to act through a stimulation of ABCA1 or ABCG5/G8 (51).

In conclusion, these results provide strong evidence for a physiological role for ABCA1 in regulating the intestinal absorption of dietary cholesterol. This “gatekeeper” role is enhanced by PPAR α activation, which results in a decreased absorption of dietary cholesterol, a decreased delivery of cholesterol to the liver, and a lower plasma concentration of total and diet-derived cholesterol. The evidence indicates that the effects of PPAR α activation on ABCA1 transcription are mediated by changes in the availability of ligands that activate LXR α , but not by changes in LXR α concentration per se. Thus, it is possible that an increase in intestinal PPAR α activation by physiological ligands such as dietary unsaturated fatty acids, particularly those of the ω -3 class (52), could contribute to the decreased intestinal uptake of cholesterol from diets rich in fish oil or oleic acid observed in nonhuman primates (53), and that this is mediated via an increased expression of ABCA1. The recent report that a specific activator of PPAR δ was a potent stimulator of ABCA1 transcription in macrophages (54) indicates that ligands for PPAR δ could also play a role in regulating cholesterol absorption. Prevention of dietary cholesterol absorption makes a significant contribution to strategies designed to reduce plasma cholesterol levels (55), so pharmacological regulation of ABCA1 activity could be important not only for reducing

the lipid content of macrophage foam cells but also for controlling the concentration of cholesterol in plasma. **PL**

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REFERENCES

- Hayden, M. R., S. M. Clee, W. A. Brooks, J. J. Genest, A. Attie, and J. J. Kastelein. 2000. Cholesterol efflux regulatory protein, Tangier disease and familial high-density lipoprotein deficiency. *Curr. Opin. Lipidol.* **11**: 117–122.
- Brooks, W. A., M. Marcil, S. M. Clee, L. H. Zhang, K. Roomp, M. van Dam, L. Yu, C. Brewer, J. A. Collins, H. O. Molhuizen, O. Loubser, B. F. Oulette, K. Fichter, E. K. Ashbourne, C. W. Sensen, S. Scherer, S. Mott, M. Denis, D. Martindale, J. Frohlich, K. Morgan, B. Koop, S. Pimstone, J. J. Kastelein, and M. R. Hayden. 1999. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat. Genet.* **22**: 336–345.
- Bodzioch, M., E. Orso, J. Klucken, T. Langmann, A. Bottcher, W. Diederich, W. Drobnik, S. Barlage, C. Buchler, O. M. Porsch, W. E. Kaminski, H. W. Hahmann, K. Oette, G. Rothe, C. Asianidis, K. J. Lackner, and G. Schmitz. 1999. The gene encoding ATP-binding cassette transporter1 is mutated in Tangier disease. *Nat. Genet.* **22**: 347–351.
- Rust, S., M. Rosier, H. Funke, J. Real, Z. Amoura, J. C. Piette, J. F. Deleuze, H. B. Brewer, N. Duverger, P. Deneffe, and G. Assmann. 1999. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter1. *Nat. Genet.* **22**: 352–355.
- Lawn, R. M., D. P. Wade, M. R. Garvin, X. Wang, K. Schwartz, J. G. Porter, J. J. Seilhamer, A. M. Vaughan, and J. F. Oram. 1999. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J. Clin. Invest.* **104**: 25–31.
- Hobbs, H. H., and D. J. Rader. 1999. ABC1: connecting yellow tonsils, neuropathy, and very low HDL. *J. Clin. Invest.* **104**: 1015–1017.
- Costet, P., Y. Luo, N. Wang, and A. R. Tall. 2000. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J. Biol. Chem.* **275**: 28240–28245.
- Repa, J. J., S. D. Turley, J. A. Lobaccaro, J. Medina, L. Li, K. Lustig, B. Shan, R. A. Heyman, J. M. Dietschy, and D. J. Mangelsdorf. 2000. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science.* **289**: 1524–1529.
- Venkateswaran, A., B. A. Laffitte, S. B. Joseph, P. A. Mak, D. C. Wilpitz, P. A. Edwards, and P. Tontonoz. 2000. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR α . *Proc. Natl. Acad. Sci. USA.* **97**: 12097–12102.
- McNeish, J., R. J. Aiello, D. Guyot, T. Turi, C. Gabel, C. Aldinger, K. L. Hoppe, M. L. Roach, L. J. Royer, J. De Wet, C. Broccardo, C. Chimini, and O. L. Francone. 2000. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc. Natl. Acad. Sci. USA.* **97**: 4245–4250.
- Drobnik, W., B. Lindenthal, B. Lieser, M. Ritter, C. T. Weber, G. Liebisch, U. Giesa, M. Igel, H. Borsukova, and C. Buchler. 2001. ATP-binding cassette transporter A1 (ABCA1) affects total body sterol metabolism. *Gastroenterology.* **120**: 1203–1211.
- Schwartz, K., R. M. Lawn, and D. P. Wade. 2000. ABC1 gene expression and ApoA-I-mediated cholesterol efflux are regulated by LXR. *Biochem. Biophys. Res. Commun.* **274**: 794–802.
- Chinetti, G., S. Lestavel, V. Bocher, A. T. Remaley, B. Neve, I. P. Torra, E. Teissier, A. Minnich, M. Jaye, N. Duverger, H. B. Brewer, J. C. Fruchart, V. Clavey, and B. Staels. 2001. PPAR- α and PPAR- α activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat. Med.* **7**: 53–58.
- Schoonjans, K., B. Staels, and J. Auwerx. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **37**: 907–925.
- Pineda Torra, I., P. Gervois, and B. Staels. 1999. Peroxisome proliferator-activated receptor α in metabolic disease, inflammation, atherosclerosis and aging. *Curr. Opin. Lipidol.* **10**: 151–159.
- Umeda, Y., Y. Kako, K. Mizutani, Y. Iikura, M. Kawamura, M. Seishima, and H. Hayashi. 2001. Inhibitory action of gemfibrozil on cholesterol absorption in rat intestine. *J. Lipid Res.* **42**: 1214–1219.
- Lee, S. S., T. Pineau, J. Drago, E. J. Lee, J. W. Owens, D. L. Kroetz, S. P. Fernandez, H. Westphal, and F. J. Gonzalez. 1995. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15**: 3012–3022.
- Peters, J. M., N. Hennuyer, B. Staels, J. C. Fruchart, C. Fievet, F. J. Gonzalez, and J. Auwerx. 1997. Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor alpha-deficient mice. *J. Biol. Chem.* **272**: 27307–27312.
- Aoyama, T., J. M. Peters, N. Iritani, T. Nakajima, K. Furihata, T. Hashimoto, and F. J. Gonzalez. 1998. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *J. Biol. Chem.* **273**: 5678–5684.
- Kersten, S., J. Seydoux, J. M. Peters, F. J. Gonzalez, B. Desvergne, and W. Wahli. 1999. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J. Clin. Invest.* **103**: 1489–1498.
- Leone, T. C., C. J. Winheimer, and D. P. Kelly. 1999. A critical role for the peroxisome proliferator-activated receptor alpha (PPAR alpha) in the cellular fasting response: the PPAR alpha-null mouse as a model of fatty acid oxidation disorders. *Proc. Natl. Acad. Sci. USA.* **96**: 7473–7478.
- Patel, D. D., B. L. Knight, D. Wiggins, S. M. Humphreys, and G. F. Gibbons. 2001. Disturbances in the normal regulation of SREBP-sensitive genes in peroxisome proliferator-activated receptor alpha (PPAR α) deficient mice. *J. Lipid Res.* **42**: 328–337.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Rosenberg, M. F., G. Verlade, R. C. Ford, C. Martin, G. Berridge, I. D. Kerr, R. Callaghan, A. Schmidlin, C. Wooding, K. J. Linton, and C. F. Higgins. 2001. Repacking of the transmembrane domains of P-glycoprotein during the transport ATPase cycle. *EMBO J.* **20**: 5615–5625.
- Schwarz, M., D. W. Russell, J. M. Dietschy, and S. D. Turley. 1998. Marked reduction in bile acid synthesis in cholesterol 7-alpha-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J. Lipid Res.* **39**: 1833–1843.
- Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–509.
- Goldfarb, S., T. A. Barber, M. W. Pariza, and D. D. Pugh. 1978. Lipid synthesis and ultrastructure of adult rat hepatocytes during their first twenty-four hours in culture. *Exp. Cell Res.* **117**: 39–46.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
- Schultz, J. R., H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwender, S. Wang, M. Thoolen, D. J. Mangelsdorf, K. D. Lustig, and B. Shan. 2000. Role of LXRs in control of lipogenesis. *Genes Dev.* **14**: 2831–2838.
- Oram, J. F., and R. M. Lawn. 2002. ABCA1: the gatekeeper for eliminating excess tissue cholesterol. *J. Lipid Res.* **42**: 1173–1179.
- Santamarina, F. S., K. Peterson, C. Knapper, Y. Qiu, L. Freeman, J. F. Cheng, J. Osorio, A. Remaley, X. P. Yang, C. Haudenschild, C. Prades, G. Chimini, E. Blackmon, T. Francois, N. Duverger, E. M. Rubin, M. Rosier, P. Deneffe, D. S. Fredrickson, and H. B. Brewer. 2000. Complete genomic sequence of the human ABCA1 gene: analysis of the human and mouse ATP-binding cassette A promoter. *Proc. Natl. Acad. Sci. USA.* **97**: 7987–7992.
- Janowski, B. A., M. J. Grogan, S. A. Jones, G. B. Wisely, S. A. Kleiwer, E. J. Corey, and D. J. Mangelsdorf. 1999. Structural requirements of ligands for the oxysterol liver X receptors LXR α and LXR β . *Proc. Natl. Acad. Sci. USA.* **96**: 266–271.
- Bjorkhem, I. 2002. Do oxysterols control cholesterol homeostasis? *J. Clin. Invest.* **110**: 725–730.
- Saucier, S. E., A. A. Kandutsch, A. K. Gayen, D. K. Swahn, and T. A. Spencer. 1989. Oxysterol regulators of 3-hydroxy-3-methylglutaryl-CoA reductase in liver. Effect of dietary cholesterol. *J. Biol. Chem.* **264**: 6863–6869.
- Edwards, P. A., H. R. Kast, and A. M. Anisfeld. 2002. BAREing it all:

the adoption of LXR and FXR and their roles in lipid homeostasis. *J. Lipid Res.* **43**: 2–12.

36. Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J. M. A. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein, and D. J. Mangelsdorf. 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXR α and LXR β . *Genes Dev.* **14**: 2819–2830.
37. Miyata, K. S., S. E. McCaw, H. V. Patel, R. A. Rachubinski, and J. A. Capone. 1996. The orphan nuclear hormone receptor LXR α interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling. *J. Biol. Chem.* **271**: 9189–9192.
38. Gbaguidi, G. F., and L. B. Agellon. 2002. The atypical interaction of peroxisome proliferator-activated receptor α with liver X receptor α antagonizes the stimulatory effect of their respective ligands on the murine cholesterol 7 α -hydroxylase gene promoter. *Biochim. Biophys. Acta.* **1583**: 229–236.
39. Ou, J., H. Tu, B. Shan, A. Luk, R. A. DeBose-Boyd, Y. Bashmakov, J. L. Goldstein, and M. S. Brown. 2001. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. *Proc. Natl. Acad. Sci. USA.* **98**: 6027–6032.
40. Johnson, T. E., and B. J. Ledwith. 2001. Peroxisome proliferators and fatty acids negatively regulate liver X receptor-mediated activity and sterol biosynthesis. *J. Steroid Biochem. Mol. Biol.* **77**: 59–71.
41. Yoshikawa, T., H. Shimano, N. Yahagi, T. Ide, M. Amemiya-Kudo, T. Matsuzaka, M. Nakakuki, S. Tomita, H. Okazaki, Y. Tamura, Y. Iizuka, K. Ohashi, A. Takahashi, H. Sone, J. Osuga, T. Gotoda, S. Ishibashi, and N. Yamada. 2002. Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements. *J. Biol. Chem.* **277**: 1705–1711.
42. Tobin, K. A. R., H. H. Steineger, S. Alberti, O. Spydevold, J. Auwerx, J.-A. Gustafsson, and H. I. Nebb. 2000. Cross-talk between fatty acid and cholesterol metabolism mediated by liver X receptor- α . *Mol. Endocrinol.* **14**: 741–752.
43. Lund, E. G., J. M. Guileyardo, and D. W. Russell. 1999. cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. *Proc. Natl. Acad. Sci. USA.* **96**: 7238–7243.
44. Brown, A. J., G. F. Watts, J. R. Burnett, R. T. Dean, and W. Jessup. 2000. Sterol 27-hydroxylase acts on 7-ketocholesterol in human atherosclerotic lesions and macrophages in culture. *J. Biol. Chem.* **275**: 27627–27633.
45. Gibbons, G. F., C. R. Pullinger, H. W. Chen, W. K. Cavenee, and A. A. Kandutsch. 1980. Regulation of cholesterol synthesis in cultured cells by probable natural precursor sterols. *J. Biol. Chem.* **255**: 375–400.
46. Gibbons, G. F., D. D. Patel, D. Wiggins, and B. L. Knight. 2002. The functional efficiency of lipogenic and cholesterogenic gene expression in normal mice and in mice lacking the peroxisomal proliferator-activated receptor α (PPAR α). *Adv. Enzyme Regul.* **42**: 227–247.
47. Patel, D. D., B. L. Knight, A. K. Soutar, G. F. Gibbons, and D. P. Wade. 2000. The effect of peroxisome-proliferator-activated receptor- α on the activity of the cholesterol 7 α -hydroxylase gene. *Biochem. J.* **351**: 747–753.
48. Post, S. M., H. Duez, P. P. Gervois, B. Staels, F. Kuipers, and H. M. G. Princen. 2001. Fibrates suppress bile acid synthesis via peroxisomal proliferator-activated receptor- α -mediated downregulation of cholesterol 7 α -hydroxylase and sterol 27-hydroxylase expression. *Arterioscler. Thromb. Vasc. Biol.* **21**: 1840–1845.
49. Brousseau, M. E. 2003. ATP-binding cassette transporter A1, fatty acids and cholesterol absorption. *Curr. Opin. Lipidol.* **14**: 35–40.
50. Repa, J. J., K. E. Berge, C. Pomajzl, J. A. Richardson, H. Hobbs, and D. J. Mangelsdorf. 2002. Regulation of ATP-binding cassette transporters ABCG5 and ABCG8 by the liver X receptors α and β . *J. Biol. Chem.* **277**: 18793–18800.
51. Repa, J. J., J. M. Dietschy, and S. D. Turley. 2002. Inhibition of cholesterol absorption by SCH 58053 in the mouse is not mediated via changes in the expression of mRNA for ABCA1, ABCG5, or ABCG8 in the enterocyte. *J. Lipid Res.* **43**: 1864–1874.
52. Devchand, P. R., H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli. 1996. The PPAR- α -leukotriene B4 pathway to inflammation control. *Nature.* **384**: 39–43.
53. Parks, J. S., and J. R. Crouse. 1992. Reduction of cholesterol absorption by dietary oleinate and fish oil in African green monkeys. *J. Lipid Res.* **33**: 559–568.
54. Oliver, W. R., J. L. Shenk, M. R. Snaith, C. S. Russell, K. D. Plunket, N. L. Bodkin, M. C. Lewis, D. A. Winegar, M. L. Sznajdman, M. H. Lambert, H. E. Xu, D. D. Sternbach, S. A. Kliewer, B. C. Hansen, and T. M. Willson. 2001. A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. *Proc. Natl. Acad. Sci. USA.* **98**: 5306–5311.
55. Turley, S. D. 2002. State of the art in cholesterol management: targeting multiple pathways. *Am. J. Manag. Care.* **8**: S29–S32.