Supporting Information

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SI Materials and Methods

Tissue Cholesterol Concentration, Synthesis, and Esters. One group of mice was studied 24 h after the injection of CYCLO at 7 days of age. They were exsanguinated, and the liver, brain, and remaining carcass were collected. Another group of mice was injected at 7 days of age and studied at 49 days of age. For these mice, the liver, brain, kidney, lung, spleen, and carcass were collected. These tissues were saponified in alcoholic potassium hydroxide (KOH), and cholesterol was extracted and quantitated by gas chromatography using stigmastanol as the internal standard (1). The tissue cholesterol levels are expressed as milligrams of cholesterol per gram of wet weight of tissue (mg/g). The total cholesterol found in every tissue was summed to give whole-animal cholesterol contents, and these values are expressed as milligrams of cholesterol per kilogram of body weight (mg/kg). The rates of cholesterol synthesis in all of the tissues were measured *in vivo* as described in ref. 1. Each animal was administered a bolus of ^3H_2O and studied 1 h later. The amount of ^3H incorporated into sterols was then determined, and these rates of cholesterol synthesis are expressed as the nanomoles of ^3H_2O incorporated into sterols per h per gram of wet weight of tissue ($nmol \cdot h^{-1} \cdot g^{-1}$). The ^3H_2O incorporation rates in all organs were summed and used to calculate the whole-animal synthesis rates. These rates were then converted to absolute milligrams of cholesterol synthesized each day per kilogram of body weight ($mg \cdot d^{-1} \cdot kg^{-1}$) (2). Rates of fatty acid synthesis in liver were determined in the same manner, and the rates are expressed as micromoles of ^3H_2O incorporated into fatty acids per h per g of wet weight of tissue ($\mu mol \cdot h^{-1} \cdot g^{-1}$). Unesterified and esterified cholesterol levels in tissues were measured as described in ref. 3 and are expressed as milligrams of unesterified or esterified cholesterol per g of wet weight tissue (mg/g).

CYCLO Pharmacokinetics. Whole-body CYCLO clearance was measured in 7- and 49-day-old mice using ^{14}C-CYCLO with an average degree of substitution of 5.15 and a specific activity of 6.95 mCi/g (Cyclodextrin Technologies Development, Inc.). The stock ^{14}C-CYCLO solution contained 1.0 mCi in 1.0 ml of water. Aliquots of the stock solution were diluted with a 20% (wt/vol) solution of nonradiolabeled CYCLO, giving ≈40,000 cpm/μL, which was used for the 7-day-old mice, or 10,000 cpm/μL, which was used for the 49-day-old mice. At each time point, 1 to 4 animals were studied. The mice were administered a s.c. injection of the ^{14}C-CYCLO solution (4,000 mg/kg) at the scruff of the neck and then were studied at various time points. Additional mice were killed immediately on completion of the injection. Such animals served as "0-time" controls. The other mice that were killed at various time points up to 24 h were returned to their cages after administration of the ^{14}C-CYCLO. At the time of study, the mice were killed, rinsed with ethanol to remove contaminating ^{14}C-CYCLO on the skin and fur, and saponified in alcoholic KOH. The carcasses were digested on a steam bath and transferred into a 100-ml flask, and the volume was brought to 100 ml with ethanol. Duplicate 1.0-ml aliquots of the liquified carcass were transferred into glass counting vials to which was added 15 ml of Cytoscint (MP Biomedicals). The total body counts, normalized to body weight, recovered from animals killed at each time point were expressed as a percentage of the normalized total baseline counts that were present in the tissues of the respective 0-time control animals. These percentage recoveries were used to calculate the proportion of the administered dose of ^{14}C-CYCLO that had been cleared from the animal over each respective time interval. These data were taken as a measure of the "whole-animal" turnover rate of ^{14}C-CYCLO in the 7- and 49-day-old mice.

Liver Function Tests. Plasma was sent to a commercial laboratory for measurement of the aspartate aminotransferase and alanine aminotransferase of the liver function tests.

Rates of Fecal Neutral Sterol and Bile Acid Excretion. Fecal neutral and acidic sterol excretion was measured as described in ref. 1. These values are expressed as milligrams of sterol excreted per day per kilogram of body weight ($mg \cdot d^{-1} \cdot kg^{-1}$).

Animal Survival Studies. The general clinical condition of the mice was monitored daily. Once the animals began to show difficulty in accessing the pelleted diet, they were also provided access to a powdered form of this diet. When mice were no longer able to take in food or water, they were humanely killed, and this was considered the day of death.

Brain Histology. Brains were removed from the animals and immersion-fixed in 10% (vol/vol) buffered formalin for 48 h. Following fixation, hindbrains (brainstem and cerebellum) were amputated from the cerebral hemispheres at the level of the superior colliculus. The cerebral hemispheres were sectioned in the coronal plane at the level of the hypothalamic infundibulum, and the hindbrains were sectioned in the axial plane at the level of the pons. Tissues were dehydrated in xylene, embedded in paraffin, and sectioned on a rotary microtome at a thickness of 3 μm. For routine histology, paraffin sections were rehydrated, stained with H&E, and evaluated by light microscopy. Purkinje cell counts were performed manually on these H&E sections in the first and second cerebellar folia in the anterior vermis represented in the axial sections, by using a calibrated ocular micrometer.

Brain Immunohistochemistry. Immunostaining was performed at room temperature on a BenchMarkXT automated immunostainer, by using the UltraVIEW system with HRP and diaminobenzidine (DAB) chromogen (Ventana Medical Systems). Optimum primary antibody dilutions were predetermined using known positive control tissues. Paraffin sections were cut at 3 μm on a rotary microtome, mounted on positively charged glass slides, and air-dried overnight. Sections were then placed onto the BenchMarkXT, where the deparaffinization and heat retrieval were performed. Sections were incubated for 1 h with primary mouse monoclonal antibody to calbindin (1:50 dilution; Novocastra) or rabbit polyclonal antibody to GFAP (1:400 dilution; Biocare) diluted in ChemMat buffer (Ventana Medical Systems) and with buffer alone as a negative reagent control. After washing in buffer, sections were then incubated with a freshly prepared mixture of DAB and H_2O_2 in buffer for 8 min, followed by washing in buffer and then water. Sections were counterstained with hematoxylin, dehydrated in a graded series of ethanols and xylene, and then coverslipped. Slides were reviewed by light microscopy. Positive reactions with DAB were identified as dark brown reaction product.

Determination of Relative mRNA Levels. Quantitative real-time PCR was performed using an Applied Biosystems 7900HT sequence detection system and SYBR-green chemistry (4). The sequences of the various primers used in these determinations

are given in Table S1. The mRNA levels are expressed relative to the housekeeping gene, cyclophilin, and are calculated by the comparative C_T method (5).

Analysis of Data. All data are presented as mean ± SEM. Differences between these mean values were tested for statistical significance ($P < 0.05$) using one-way ANOVA, followed by the Newman-Keuls multiple comparison test (GraphPad Software, Inc.). Significant differences between groups are designated with different letters in the various figures. Significant differences in survival curves were determined by the Wilcoxon-Gehar and log-rank analysis.

1. Schwarz M, Russell DW, Dietschy JM, Turley SD (1998) Marked reduction in bile acid synthesis in cholesterol 7α-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J Lipid Res* 39:1833–1843.
2. Dietschy JM, Spady DK (1984) Measurement of rates of cholesterol synthesis using tritiated water. *J Lipid Res* 25:1469–1476.
3. Beltroy EP, Liu B, Dietschy JM, Turley SD (2007) Lysosomal unesterified cholesterol content correlates with liver cell death in murine Niemann-Pick type C disease. *J Lipid Res* 48:869–881.
4. Kurrasch DM, Huang J, Wilkie TM, Repa JJ (2004) Quantitative real-time polymerase chain reaction measurement of regulators of G protein signaling mRNA levels in mouse tissues. *Methods Enzymol* 389:3–15.
5. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C_T method. *Nature Protocols* 3:1101–1108.

Table S1. PCR primers used to quantify mRNA levels

Gene	Full name	Accession number	Sequence of primers (5′ to 3′)
ABCA1	ATP-binding cassette A1	NM_013454	F: CGTTTCCGGGAAGTGTCCTA R: GCTAGAGATGACAAGGAGGATGGA
ABCG1	ATP-binding cassette G1	NM_009593	F: GCTGTGCGTTTTGTGCTGTT R: TGCAGCTCCAATCAGTAGTCCTAA
ABCG5	ATP-binding cassette G5	NM_031884	F: TGGATCCAACACCTCTATGCTAAA R: GGCAGGTTTTCTCGATGAACTG
ABCG8	ATP-binding cassette G8	NM_026180	F: TGCCCACCTTCCACATGTC R: ATGAAGCCGGCAGTAAGGTAGA
ApoD	Apolipoprotein D	NM_007470	F: TCCAGTTCTTCCCGTTGATG R: CGAGGGCATAGTTTTCATAATCG
MIP-1α (CCL3)	Chemokine ligand 3	NM_011337	F: TTCATCGTTGACTATTTTGAAACCA R: GCCGGTTTCTCTTAGTCAGGA
CD68	CD68 antigen	NM_009853	F: CCTCCACCCTCGCCTAGTC R: TTGGGTATAGGATTCGGATTTGA
CYP7A1	Cytochrome P450 7A1	NM_007824	F: AGCAACTAAACAACCTGCCAGTACTA R: GTCCGGATATTCAAGGATGCA
HMG CoA RED	3-hydroxy-3-methylglutaryl coenzyme A reductase	NM_008255	F: CTTGTGGAATGCCTTGTGATTG R: AGCCGAAGCAGCACATGAT
HMG CoA SYN	3-hydroxy-3-methylglutaryl coenzyme A synthase 1	NM_145942	F: GCCGTGAACTGGGTCGAA R: GCATATATAGCAATGTCTCCTGCAA
CD11c (ITGAX)	Integrin alpha X	NM_021334	F: CTTCATTCTGAAGGGCAACCT R: CACTCAGGAGCAACACCTTTTT
LDLR	Low-density lipoprotein receptor	NM_010700	F: GAGGAACTGGCGGCTGAA R: GTGCTGGATGGGGAGGTCT
ACAT2 (SOAT2)	Sterol O-acyltransferase 2	NM_146064	F: GCCTCCGCCGAGATGCT R: GTAGTTGGAGAAGGAAGTCGAGTTC
SREBP2 (SREBF2)	Sterol regulatory element 2	NM_033218	F: ACAAACTTGCTCTGAAAACAAATC R: GCGTTCTGGAGACCATGGA
TLR4	Toll-like receptor 4	NM_021297	F: ACTGTTCTTCTCCTGCCTGACA R: TGATCCATGCATTGGTAGGTAATA
TNF-α	Tumor necrosis factor-α	NM_013693	F: CTGAGGTCAATCTGCCCAAGTAC R: CTTCACAGAGCAATGACTCCAAAG

F, forward; R, reverse.