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ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 545–554
Journal of Nutritional Biochemistry

Long-term phytosterol treatment alters gene expression in the liver of apo E-deficient mice

Zuyuan Xu, Khuong Le, Mohammed H. Moghadasian*

Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Canada R2H 2A6
Canadian Center for Agri-food Research in Health and Medicine, St. Boniface Hospital Research Center, Winnipeg, Canada R2H 2A6

Received 29 November 2006; received in revised form 13 June 2007; accepted 20 June 2007

Abstract

Dietary phytosterols significantly reduce plasma cholesterol concentrations and atherosclerosis in apolipoprotein E-knockout (apo E-KO) mice. We investigated the long-term effects of phytosterol treatment on gene expression in the liver of these mice. Male apo E-KO mice were fed an atherogenic diet supplemented with (n=6) or without (n=6) 2% (wt/wt) phytosterol mixtures for 14 weeks. Liver specimens were collected and stored in RNAlater immediately. mRNA was extracted and subjected to microarray analyses and real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) assay for confirmation. Oligonuleotide microarray analysis of pooled samples (n=3) revealed that the expression of 132 genes/transcripts was significantly altered in treated animals, considering the false discovery rate (FDR) of 0.23. Real-time RT-PCR techniques confirmed these alterations in the expression of several of these genes, including *Hmgcr* (2.16-fold; P=.0002), *Hmgcs1* (1.79-fold; P=.001), *Hsd17b7* (2.11-fold; P=.028), *Sqle* (2.03-fold; P=.01), *Cyp51* (1.8-fold; P=.001), *Fads1* (1.55-fold; P=.031), *Fads2* (2.17-fold; P=.047), *Lpin1* (3.67-fold; P=.001), *Ppargc1b* (PGC-1β; a coactivator of sterol-regulatory element-binding proteins; 1.66-fold; P=.007) and *Cyp7B1* (1.81-fold; P=.025). In summary, our data suggest that long-term dietary phytosterols can alter the expression of a number of hepatic genes that regulate sterol metabolism in apo E-KO mice. It is possible that these changes are due to inhibition of cholesterol absorption, but are not a direct effect of plant sterols. Further multivariate correlation or association analysis is needed to establish the relations between changes in the expression of these genes and prevention of atherosclerosis by phytosterols.

Keywords: Apo E-KO mice; Atherosclerosis; Gene expression; Liver; Microarray; Phytosterols

1. Introduction

Atherosclerosis is a multifactorial disease that causes coronary artery disease — a major cause of mortality and morbidity worldwide. Among several modifiable risk factors, increased levels of low-density lipoprotein (LDL) cholesterol are well-associated with progression of the disease. Over 50 years ago, phytosterols were found to lower serum cholesterol in animals and humans [1,2]. Phytosterols are commonly found in nuts, vegetable oils and other fatty fruits and vegetables [3]. It is now well established that consumption of plant sterols through enriched food products can moderately reduce LDL-cholesterol concentrations [4]. In fact, such phytosterol-enriched food products have been marketed as cholesterol-lowering functional foods in >20 countries [5]. Despite the overt availability and consumption of these functional foods worldwide, the long-term clinical benefits of plant sterol consumption on the prevention of the progression or induction of atherosclerotic lesions regression in humans have not been documented.

We have consistently shown that addition of a 2% (wt/wt) phytosterol mixture to the diet of apolipoprotein E-knockout (apo E-KO) mice significantly reduces plasma cholesterol levels and prevents aortic atherosclerosis [6–10]. The exact mechanisms of these effects are not known. Phytosterols are not systemically absorbed, but they inhibit the absorption of cholesterol, including recirculating endogenous biliary cholesterol [2]. Therefore, several laboratories have attempted to establish the mechanisms of inhibition of intestinal cholesterol absorption by dietary phytosterols [11,12]. In this

* Corresponding author. Canadian Center for Agri-food Research in Health and Medicine, Winnipeg, MB, Canada R2H 2A6. Tel.: +1 204 235 3934; fax: +1 204 231 1151.
E-mail address: mmoghadasian@sbrc.ca (M.H. Moghadasian).

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doi:10.1016/j.jnutbio.2007.06.012

regard, displacement of cholesterol by phytosterols from mixed micelles is one of the original hypotheses for the cholesterol-lowering effects of phytosterols [12]. Recent studies have suggested a role for ATP-binding cassette (ABC) transporters G5, G8 and A1 for cholesterol and phytosterol intestinal absorption in humans [13,14]. Mutations in *ABCG5* and *ABCG8* are associated with increased phytosterol absorption and development of phytosterolemia in humans [15,16]. Furthermore, deletions of these genes in experimental animals resulted in increased phytosterol absorption in mice [17]. Other studies have shown that inhibition of intestinal cholesterol absorption is not mediated via transcriptional changes in *ABCA1*, *ABCG5* and *ABCG8* [18]. However, we did not observe significant alterations in the expression of *ABCG5*, *ABCG8* and *ABCA1* in the small intestine of phytosterol-treated apo E-KO mice, as compared to controls [19]. Instead, we observed an overexpression of acyl-coenzyme-A-binding protein, cellular-retinol-binding protein II, cytochrome *b* and Claudin 7 in the small intestine of the phytosterol-treated apo E-KO mice, as compared to corresponding controls [19].

The cholesterol-lowering effects of plant sterols are well-established in both human and animal models [6–10,20–22]. Inhibition of intestinal cholesterol absorption may change liver sterol metabolism [23]. This phenomenon can be investigated through identification of alterations in hepatic gene expression after long-term phytosterol treatment in a responsive animal model. Thus, the aim of this study was to utilize our previous experiences with phytosterol therapy in apo E-KO mice and to determine alterations in hepatic gene expression following long-term administration of dietary phytosterols using cDNA microarray techniques. Phytosterol treatment was associated with significant alterations in the expression of 132 genes [false discovery rate (FDR)=0.23] involved in different biological processes. Among them, genes related to steroid metabolism, especially cholesterol synthesis and bile acid synthesis, were significantly affected. These observations may suggest mechanisms for maintaining the normal cholesterol homeostasis necessary for physiological functions of cells.

2. Materials and methods

2.1. Animals and diets

Twelve 4-week-old apo E-KO mice were used in this study. The animals were purchased from the Jackson Laboratory and housed in a humidity- and temperature-controlled room with a 12-h dark/12-h light cycle and ad libitum access to food and water. We have consistently used young apo E-KO mice [6–10] because these animals develop spontaneous atherosclerosis; this phenomenon may interfere with diet therapy if older animals are used. After a 10-day adaptation period, they were assigned to control (n=6) or phytosterol-treated (n=6) groups matched for average body weight and plasma cholesterol concentrations, as previously described [6–10]. A PicoLab mouse diet containing protein (20.5% wt/wt), fat (9% wt/wt), cholesterol (285 ppm), carbohydrate (53% wt/wt), ash and vitamins (4.8% wt/wt), fiber (2.7% wt/wt) and moisture (10%, wt/wt) was supplemented with 0.2% (wt/wt) cholesterol (Sigma-Aldrich Canada Ltd., Oakville, Ontario) for the control group; this "atherogenic diet" was further supplemented with 2% (wt/wt) phytosterol mixture containing 58% β-sitosterol, 19% campesterol, 13% dihydrobrassicasterol and 10% stigmasterol (Sigma-Aldrich Canada Ltd.) and used for the treated group. The experiment was carried out over 14 weeks. At sacrifice, blood samples were taken from the right ventricle, and liver samples were collected and stored in RNAlater (Ambion, Inc., Austin, TX). The aorta and heart were harvested and fixed for histological examinations. All animal protocols were approved by the Animal Care Committee at the University of Manitoba.

2.2. Plasma cholesterol levels and aortic atherosclerosis

Standard enzymatic assays for cholesterol measurement, as well as routine histological techniques for assessment of atherosclerotic lesions, were used. We have extensively described these methods in this animal model elsewhere [6–10].

2.3. Affymetrix oligonucleotide microarrays assay

Total RNA was extracted from liver tissues using Trizol reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's instructions. RNAs were purified using Qiagen RNeasy MinElute Cleanup Kit (Qiagen, Inc., Mississauga, Ontario) in accordance with the manufacturer's instructions. RNA samples from two mice of each experimental group were pooled together to generate three samples from the phytosterol-treated group and three samples from the control group and used for microarray assays at the Microarray Facility of the Hospital for Sick Children (Toronto, Canada) in accordance with the manufacturer's instructions. The Affymetrix GeneChip Mouse Genome 430 2.0 Array containing 45,037 probe sets and >39,000 transcripts was used (www.affymetrix.com).

2.4. Microarray data analysis

Hybridized gene microarrays were scanned by Affymetrix GeneChip Scanner 3000, and raw data were normalized using Robust Multichip Average algorithm [24], which is implemented in the Affymetrix package of the Bioconductor microarray analysis software [25]. Differentially expressed genes were identified using nonspecific gene-filtering "present/marginal/absent" (P/M/A) calls algorithm [26,27], followed by the application of the significance analysis of microarrays (SAM) software [28]. SAM software is a statistical tool that was developed for finding differentially expressed genes in microarray experiments (http://www-stat.stanford.edu/~tibs/SAM/). SAM assigns a score to each gene based on changes in gene expression relative to the standard deviation of repeated measurements. For genes with scores greater than an adjustable threshold, SAM uses permutations

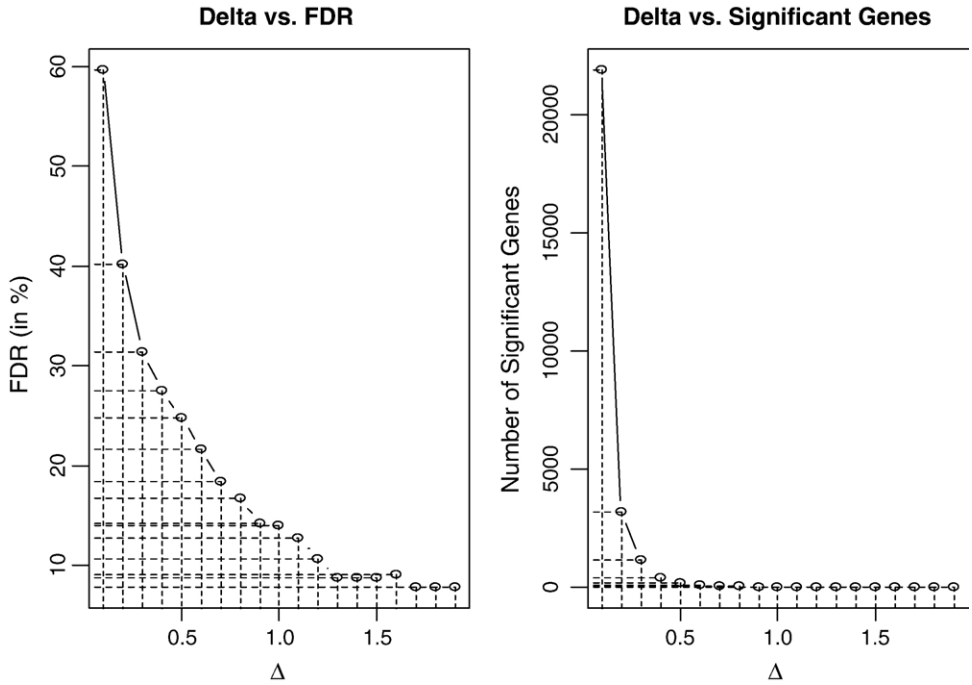

Fig. 1. The relationship between SAM Δ, FDR and the number of significant genes in this microarray assay (n=3 in each group). Considering this relationship and Δ=0.6 and FDR=0.23, 132 genes showed significant alterations in their expression in the treated group as compared to the control group. (Parameters for SAM analysis: number of permutations, 100; Pooler variance was used to compute for t statistics; default values were used for other parameters.)

of the repeated measurements to estimate the percentages of falsely identified genes (FDR) [28].

2.5. Biological pathway analysis

We identified the functions of significantly expressed genes by mapping them to the Gene Ontology database (www.geneontology.org), as previously described [29]. Gene Set Enrichment Analysis (GSEA) was used to analyze the biological pathways of those differentially expressed genes. This approach evaluates microarray data at the level of gene sets and is more powerful than conventional strategies that focus on identifying individual differentially expressed genes (http://www.broad.mit.edu/gsea/) [30]. GenMAPP V.2.0 and MAPPFinder V.2.0 (http://www.genmapp.org) were also used to further identify the pathways of genes with significant alterations in their expression, as previously described [31].

2.6. Real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) analyses

The purified total RNA from all of the liver specimens (n=6 in each group) was reverse transcribed with Oligo(dT) using SuperScript II reverse transcriptase (Invitrogen Canada, Inc., Burlington, Ontario) to generate cDNA. Real-time quantitative RT-PCR analyses were performed using the Applied Biosystems Prism 7500 real-time PCR system (Applied Biosystems, Foster, CA) in accordance with the manufacturer's protocol. The PCR contained (final volume, 25 μl) 30 ng of reverse-transcribed RNA, 12.5 μl of 2× SYBR Green PCR Master Mix (Applied Biosystems) and a 100-nM concentration for each primer. Genes were amplified with 1 cycle of 50°C for 2 min, 1 cycle of 95°C for 10 min and 40 cycles of 95°C for 15 s, 55°C for 25 s and 60°C for 35 s, with each gene being tested in triplicate, and β-actin was used as an internal control. The data were analyzed using 7500 system sequence detection software (version 1.3.1; Applied Biosystems) to determine the relative quantitative gene expression.

2.7. Statistical analysis

For the analysis of microarray data, SAM software (http://www-stat.stanford.edu/~tibs/SAM/) was used to determine statistically significant changes in the expression of genes given the values of FDR=0.23 and q=0.25, as previously described [28]. Student's t test was also used to determine significant changes in the expression of genes following real-time RT-PCR procedures in the treated group as compared to controls.

3. Results

3.1. Plasma cholesterol levels and aortic atherosclerosis

As we have reported previously [6–10], consumption of dietary phytosterols significantly reduced plasma cholesterol concentrations and the extent and severity of atherosclerotic lesions in the aortic roots of these animals. Due to demonstrations of such results in previous publications [6–10], these data are not included in this report.

Table 1
Significant alterations in hepatic gene expression following long-term treatment with phytosterols in apo E-KO mice

	Probe ID	Gene title	Gene symbol	Gene Ontology biological process	*d*	*q*	Fold change
1	1417168_a_at	Ubiquitin-specific peptidase 2	*Usp2*	Ubiquitin-dependent protein catabolism	−2.72	0.2	2.25
2	1429399_at	Ring finger protein 125	*Rnf125*	Ubiquitin cycle	2.55	0.21	0.69
3	1458176_at	Period homolog 3 (*Drosophila*)	*Per3*	Two-component signal transduction system (phosphorelay)	−3.64	0.15	2.02
4	1457350_at	Period homolog 2 (*Drosophila*)	*Per2*	Two-component signal transduction system (phosphorelay)	−3.1	0.17	1.53
5	1449851_at	Period homolog 1 (*Drosophila*)	*Per1*	Two-component signal transduction system (phosphorelay)	−2.65	0.2	1.64
6	1416108_a_at	Transmembrane emp24 domain containing 3	*Tmed3*	Transport	2.51	0.22	0.64
7	1444185_at	Stromal interaction molecule 2	*Stim2*	Transport	−2.44	0.23	1.33
8	1434096_at	Solute carrier family 4 (anion exchanger), member 4	*Slc4a4*	Transport	3.04	0.18	0.65
9	1437990_x_at	Hemoglobin Z, β-like embryonic chain	*Hbb-bh1*	Transport	2.44	0.23	0.7
10	1455269_a_at	Coronin, actin-binding protein 1A	*Coro1a*	Transport	3.15	0.17	0.45
11	1424344_s_at	Eukaryotic translation initiation factor 1A	*Eif1a*	Translational initiation	2.94	0.18	0.65
12	1425837_a_at	CCR4 carbon catabolite repression 4-like (*Saccharomyces cerevisiae*)	*Ccrn4l*	Transcription from RNA polymerase II promoter	−4.83	0.14	2.86
13	1451716_at	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)	*Mafb*	Transcription	−2.4	0.24	1.72
14	1424175_at	Thyrotroph embryonic factor	*Tef*	Transcription	−3.84	0.14	1.74
15	1460035_at	Prohibitin 2	*Phb2*	Transcription	−2.29	0.25	1.32
16	1449945_at	Peroxisome-proliferator-activated receptor, γ, coactivator 1 β	*Ppargc1b* (PGC-1β)	Transcription	−2.62	0.2	1.36
17	1416958_at	Nuclear receptor subfamily 1, group D, member 2	*Nr1d2*	Transcription	−2.7	0.2	1.62
18	1438211_s_at	D-site albumin-promoter-binding protein	*Dbp*	Transcription	−3.85	0.14	5.39
19	1418025_at	Basic helix–loop–helix domain containing, class B2	*Bhlhb2*	Transcription	−2.73	0.2	2.03
20	1427127_x_at	Heat shock protein 1B	*Hspa1b*	Telomere maintenance	−3.84	0.14	3.77
21	1452388_at	Heat shock protein 1A	*Hspa1a*	Telomere maintenance	−5.96	0.14	2.42
22	1423804_a_at	Isopentenyl-diphosphate δ isomerase	*Idi1*	Steroid biosynthesis	−2.78	0.19	2.05
23	1417871_at	Hydroxysteroid (17-β) dehydrogenase 7	*Hsd17b7*	Steroid biosynthesis	−2.34	0.24	1.6
24	1459497_at	Farnesyl diphosphate farnesyl transferase 1	*Fdft1*	Steroid biosynthesis	−2.35	0.24	1.54
25	1426808_at	Lectin, galactose binding, soluble 3	*Lgals3*	Skeletal development	4.24	0.14	0.51
26	1434620_s_at	RIKEN cDNA 2610024E20 gene	*2610024E20Rik*	Signal transduction	2.49	0.22	0.68
27	1420699_at	C-type lectin domain family 7, member a	*Clec7a*	Response to yeast	3.42	0.17	0.63
28	1424737_at	Thyroid-hormone-responsive SPOT14 homolog (*Rattus*)	*Thrsp*	Regulation of transcription from RNA polymerase II promoter	−2.44	0.23	2.88
29	1418300_a_at	MAP-kinase-interacting serine/threonine kinase 2	*Mknk2*	Regulation of protein biosynthesis	−2.36	0.24	1.43
30	1448667_x_at	Transducer of ERBB2, 2	*Tob2*	Regulation of progression through the cell cycle	−2.99	0.18	2.04
31	1440481_at	Signal transducer and activator of transcription 1	*Stat1*	Regulation of progression through the cell cycle	2.35	0.24	0.73
32	1421375_a_at	S100 calcium-binding protein A6 (calcyclin)	*S100a6*	Regulation of progression through the cell cycle	2.66	0.2	0.41
33	1419123_a_at	Platelet-derived growth factor, C polypeptide	*Pdgfc*	Regulation of progression through the cell cycle	2.7	0.2	0.53
34	1422439_a_at	Cyclin-dependent kinase 4	*Cdk4*	Regulation of progression through the cell cycle	2.29	0.25	0.72
35	1418918_at	Insulin-like growth-factor-binding protein 1	*Igfbp1*	Regulation of cell growth	2.47	0.22	0.38
36	1452661_at	Transferrin receptor	*Tfrc*	Proteolysis	2.45	0.23	0.68
37	1425099_a_at	Aryl hydrocarbon receptor nuclear translocator-like	*Arntl*	Protein import into the nucleus	3.25	0.17	0.47
38	1423447_at	Caseinolytic peptidase X (*Escherichia coli*)	*Clpx*	Protein folding	3.38	0.17	0.51
39	1459808_at	FK506-binding protein 4	*Fkbp4*	Protein folding	−2.64	0.2	1.45

Table 1 (*continued*)

	Probe ID	Gene title	Gene symbol	Gene Ontology biological process	*d*	*q*	Fold change
40	1447803_x_at	Capping protein (actin filament), gelsolin-like	*Capg*	Protein complex assembly	2.68	0.2	0.64
41	1448370_at	Unc-51 like kinase 1 (*Caenorhabditis elegans*)	*Ulk1*	Protein amino acid phosphorylation	−2.85	0.19	1.47
42	1456226_x_at	Discoidin domain receptor family, member 1	*Ddr1*	Protein amino acid phosphorylation	2.94	0.18	0.72
43	1418946_at	ST3 β-galactoside α-2,3-sialyltransferase 1	*St3gal1*	Protein amino acid glycosylation	3.68	0.15	0.65
44	1456147_at	ST8 α-*N*-acetyl-neuraminide α-2,8-sialyltransferase 6	*St8sia6*	Protein amino acid glycosylation	−2.54	0.21	1.36
45	1452783_at	Fibronectin-type III domain-containing 3B	*Fndc3b*	Positive regulation of fat cell differentiation	3.12	0.17	0.64
46	1443849_x_at	Uroporphyrinogen decarboxylase	*Urod*	Porphyrin biosynthesis	2.36	0.24	0.73
47	1449049_at	Toll-like receptor 1	*Tlr1*	Physiological defense response	2.54	0.21	0.64
48	1416086_at	Protein-tyrosine sulfotransferase 2	*Tpst2*	Peptidyl-tyrosine sulfation	2.83	0.19	0.69
49	1460059_at	Uridine phosphorylase 2	*Upp2*	Nucleoside metabolism	−2.69	0.2	1.5
50	1457670_s_at	Lamin A	*Lmna*	Nuclear membrane organization and biogenesis	3.51	0.17	0.54
51	1447845_s_at	Vanin 1	*Vnn1*	Nitrogen compound metabolism	3.1	0.17	0.47
52	1419185_a_at	MLX-interacting protein-like	*Mlxipl*	Negative regulation of transcription from RNA polymerase II promoter	−2.29	0.25	1.43
53	1421515_at	Nuclear receptor subfamily 6, group A, member 1	*Nr6a1*	Negative regulation of transcription from RNA polymerase II promoter	−2.58	0.21	1.39
54	1416029_at	Kruppel-like factor 10	*Klf10*	Negative regulation of transcription from RNA polymerase II promoter	−2.43	0.23	2.13
55	1416630_at	Inhibitor of DNA binding 3	*Id3*	Negative regulation of transcription from RNA polymerase II promoter	2.41	0.24	0.59
56	1427347_s_at	Tubulin, β 2a	*Tubb2a*	Microtubule-based process	2.36	0.24	0.59
57	1423397_at	UDP glucuronosyltransferase 2 family, polypeptide B38	*Ugt2b38*	Metabolism	−2.66	0.2	2.65
58	1416181_at	Mesoderm development candidate 2	*Mesdc2*	Mesoderm development	2.41	0.24	0.76
59	1418028_at	Dopachrome tautomerase	*Dct*	Melanin biosynthesis from tyrosine	−2.73	0.2	1.87
60	1420760_s_at	N-*myc* downstream regulated-like	*Ndrl*	Mast cell activation	2.95	0.18	0.49
61	1456174_x_at	N-*myc* downstream-regulated gene 1	*Ndrg1*	Mast cell activation	3.06	0.18	0.45
62	1424518_at	RIKEN cDNA 2310016F22 gene	*2310016F22Rik*	Lipid transport	2.6	0.21	0.67
63	1427229_at	3-Hydroxy-3-methylglutaryl-coenzyme A reductase	*Hmgcr*	Lipid metabolism	−2.56	0.21	2.18
64	1418288_at	Lipin 1	*Lpin1*	Lipid metabolism	−3.07	0.18	2.97
65	1440444_at	Fatty acid desaturase 1	*Fads1*	Lipid metabolism	−2.91	0.18	1.88
66	1450264_a_at	Choline kinase α	*Chka*	Lipid metabolism	3.43	0.17	0.42
67	1450884_at	CD36 antigen	*Cd36*	Lipid metabolism	3.09	0.17	0.47
68	1448756_at	S100 calcium-binding protein A9 (calgranulin B)	*S100a9*	Leukocyte chemotaxis	2.52	0.21	0.25
69	1421345_at	Lecithin-retinol acyltransferase	*Lrat*	Lecithin metabolism	−2.39	0.24	1.46
70	1458758_at	Vav2 oncogene	*Vav2*	Intracellular signaling cascade	−2.33	0.24	1.3
71	1416846_a_at	PDZ domain-containing RING finger 3	*Pdzrn3*	Intracellular signaling cascade	3.316	0.17	0.5
72	1448748_at	Pleckstrin	*Plek*	Intracellular signaling cascade	2.57	0.21	0.56
73	1426663_s_at	Solute carrier family 45, member 3	*Slc45a3*	Inferred from electronic annotation	2.55	0.21	0.59
74	1449009_at	T-cell-specific GTPase	*Tgtp*	Immune response	3.14	0.17	0.49
75	1426037_a_at	Regulator of G-protein signaling 16	*Rgs16*	G-protein-coupled receptor protein signaling pathway	−2.73	0.2	2.71
76	1428070_at	Synovial apoptosis inhibitor 1, synoviolin	*Syvn1*	Embryonic development (sensu Mammalia)	3.13	0.17	0.61
77	1421075_s_at	Cytochrome *P*450, family 7, subfamily b, polypeptide 1	*Cyp7B1*	Electron transport	−2.65	0.2	1.67
78	1422100_at	Cytochrome *P*450, family 7, subfamily a, polypeptide 1	*Cyp7a1*	Electron transport	−2.56	0.21	2.43
79	1422533_at	Cytochrome *P*450, family 51	*Cyp51*	Electron transport	−2.32	0.24	1.79
80	1415993_at	Squalene epoxidase	*Sqle*	Electron transport	−2.2	0.25	1.8
81	1428636_at	Six-transmembrane epithelial antigen of prostate 2	*Steap2*	Electron transport	2.29	0.25	0.72

(*continued on next page*)

Table 1 (*continued*)

	Probe ID	Gene title	Gene symbol	Gene Ontology biological process	*d*	*q*	Fold change
82	1419031_at	Fatty acid desaturase 2	*Fads2*	Electron transport	−2.4	0.24	1.6
83	1450484_a_at	Thymidylate kinase family lipopolysaccharide-inducible member	*Tyki*	dTDP biosynthesis	2.55	0.21	0.67
84	1428547_at	5′ nucleotidase, ecto	*Nt5e*	DNA metabolism	3.83	0.14	0.57
85	1421430_at	RAD51-like 1 (*S. cerevisiae*)	*Rad51l1*	DNA metabolism	4.24	0.14	0.3
86	1416746_at	H2A histone family, member X	*H2afx*	DNA damage checkpoint	2.38	0.24	0.73
87	1436902_x_at	Thymosin, β 10	*Tmsb10*	Cytoskeleton organization and biogenesis	3.26	0.17	0.54
88	1419394_s_at	S100 calcium-binding protein A8 (calgranulin A)	*S100a8*	Chemotaxis	2.58	0.21	0.23
89	1440847_at	Metastasis suppressor 1	*Mtss1*	Cell motility	−2.55	0.21	2.06
90	1417377_at	Immunoglobulin superfamily, member 4A	*Igsf4a*	Cell adhesion	2.32	0.24	0.71
91	1448303_at	Glycoprotein (transmembrane) nmb	*Gpnmb*	Cell adhesion	3.22	0.17	0.57
92	1452445_at	Solute carrier family 41, member 2	*Slc41a2*	Cation transport	2.54	0.21	0.57
93	1426523_a_at	Glucosamine-6-phosphate deaminase 2	*Gnpda2*	Carbohydrate metabolism	2.3	0.25	0.75
94	1450048_a_at	Isocitrate dehydrogenase 2 ($NADP^+$), mitochondrial	*Idh2*	Carbohydrate metabolism	2.66	0.2	0.71
95	1460196_at	Carbonyl reductase 1	*Cbr1*	Biological process	2.62	0.2	0.59
96	1457985_at	Calmin	*Clmn*	Biological process	−2.65	0.2	1.39
97	1422614_s_at	Biogenesis of lysosome-related organelles complex 1, subunit 1	*Bloc1s1*	Biological process	2.97	0.18	0.74
98	1428112_at	Arginine-rich, mutated in early stage tumors	*Armet*	Biological process	3.63	0.15	0.53
99	1435834_at	Growth arrest specific 2	*Gas2*	Apoptosis	2.61	0.2	0.75
100	1460251_at	FAS (TNF receptor superfamily member)	*FAS*	Apoptosis	2.66	0.2	0.63
101	1419004_s_at	B-cell leukemia/lymphoma-2-related protein A1a	*Bcl2a1a*	Apoptosis	2.57	0.21	0.67
102	1433445_x_at	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1	*Hmgcs1*	Acetyl-CoA metabolism	−2.85	0.18	1.61
103	1420875_at	Twinfilin, actin-binding protein, homolog 1 (*Drosophila*)	*Twf1*	–	2.39	0.24	0.76
104	1420123_at	T-cell leukemia translocation altered gene	*Tcta*	–	2.33	0.24	0.77
105	1417654_at	Syndecan 4	*Sdc4*	–	2.31	0.24	0.52
106	1424524_at	RIKEN cDNA 1200002N14 gene	*1200002N14Rik*	–	2.83	0.19	0.72
107	1451313_a_at	RIKEN cDNA 1110067D22 gene	*1110067D22Rik*	–	3.87	0.14	0.56
108	1450914_at	Protein phosphatase 1, regulatory (inhibitor) subunit 14B	*Ppp1r14b*	–	2.47	0.23	0.71
109	1437226_x_at	MARCKS-like 1	*Marcksl1*	–	3.13	0.17	0.65
110	1460290_at	Lipin 2	*Lpin2*	–	−3.12	0.17	1.56
111	1417141_at	Interferon-γ-induced GTPase	*Igtp*	–	2.66	0.2	0.66
112	1422787_at	FK506-binding protein-like	*Fkbpl*	–	2.28	0.25	0.7
113	1416326_at	Cysteine-rich protein 1 (intestinal)	*Crip1*	–	2.44	0.23	0.67
114	1460510_a_at	Coenzyme Q10 homolog B (*S. cerevisiae*)	*Coq10b*	–	−2.76	0.2	1.78
115	1457619_at	cDNA sequence BC015286	*BC015286*	–	−3.63	0.15	2.16
116	1460218_at	CD52 antigen	*Cd52*	–	3.31	0.17	0.66
117	1459865_x_at	Carboxylesterase 7	*Ces7*	–	−2.32	0.24	1.31
118++	1429184_at	GTPase, very large interferon-inducible 1	*Gvin1*	–	3.11	0.17	0.56
119++	1446219_at	RIKEN cDNA D930015E06 gene	*D930015E06Rik*	–	−2.94	0.18	1.39
120++	1417161_at	CDK2-associated protein 2	*Cdk2ap2*	–	2.94	0.18	0.6
121++	1436194_at	RIKEN cDNA C330008K14 gene	*C330008K14Rik*	–	2.89	0.18	0.62
122++	1454460_at	RIKEN cDNA 5730433N10 gene	*5730433N10Rik*	–	−3.2	0.17	1.66
123++	1428126_a_at	RIKEN cDNA 4921506J03 gene	*4921506J03Rik*	–	2.487	0.22	0.72
124++	1426223_at	RIKEN cDNA 2810439F02 gene	*2810439F02Rik*	–	−2.76	0.2	1.5
125++	1428325_at	RIKEN cDNA 2610019P18 gene	*2610019P18Rik*	–	2.33	0.24	0.75
126++	1428196_a_at	RIKEN cDNA 1200015F23 gene	*1200015F23Rik*	–	2.45	0.23	0.75
127++	1445574_at	Transcribed locus	–	–	−2.7	0.2	1.63
128++	1419857_at	Transcribed locus	–	–	−2.38	0.24	1.48
129++	1424969_s_at	–	–	–	−4.68	0.14	2.88
130++	1451548_at	–	–	–	−4.43	0.14	3.29
131++	1442537_at	–	–	–	−3.01	0.18	1.47
132++	1427820_at	–	–	–	−2.35	0.24	1.83

Positive *d*-values indicate down-regulated genes and negative *d*-values indicate up-regulated genes in phytosterol-treated mice as compared to controls. FDR=0.23 (q=0.25).

(++) EST or genes/transcripts with unknown function.

3.2. Hepatic global gene expression change profile

Fig. 1 shows the relationships between Δ and FDR, and between Δ and the number of significantly altered genes in this study. This relationship indicates that no significant alteration in gene expression can be identified when FDR≤0.14 (corresponding to a Δ value of approximately 1.1 and a *q*-value of 0.14). Because of the small sample size (*n*=3 for each group), we raised the cutoff values of FDR to 0.23 (*q*=0.25) to be able to identify genes with significant alterations. Using this system, we identified 132 genes/transcripts with significant alterations in their expression. Among them, the expression of 57 genes was up-regulated, and the remaining 75 genes demonstrated down-regulated expression. Gene annotation analysis revealed that 117 of these genes have known function. The remaining 15 genes are expressed sequence tags (EST) or genes/transcripts with unknown function (Table 1). Annotation of the Gene Ontology biological process indicated that five genes are involved in lipid metabolism (*Fads1*, *Chka*, *Cd36*, *Lpin1* and *Hmgcr*), three genes are involved in steroid biosynthesis (*Idi1*, *Hsd17b7* and *Fdft1*), one gene is involved in acetyl-CoA metabolism (*Hmgcs1*), six genes are involved in electron transport (*Cyp7a1*, *Cyp7B1*, *Cyp51*, *Fads2*, *Sqle* and *Steap2*) and seven genes are involved in transcription, including *Ppargc1b* (PGC-1β); this information is summarized in Table 1. Further GSEA and GenMAPP (MAPPFinder) studies revealed that sterol metabolism (*Z*=15.3, *P*=.04), especially cholesterol metabolism, was markedly affected by phytosterol therapy (*Z*=14.3, *P*=.04).

3.3. Expression of genes related to sterol metabolism and adipogenesis in the liver using real-time quantitative RT-PCR

Sixteen of the genes with significant alterations in their expression (13 genes for sterol metabolism and 3 genes for adipogenesis and apoptosis) were further tested by real-time quantitative RT-PCR assay using the original samples (*n*=6 in each group). The mRNA levels of 12 genes tested by real-time RT-PCR were almost the same as those observed in microarray assay data, while 4 genes (*Cyp7a1*, *Fdft1*, *Fkbp4* and *Id3*) did not show significant alterations in their expression. Thus, the calculated value of FDR confirmed by real-time RT-PCR is 0.25 (4/16).

Table 2 shows the expression levels of 13 genes of sterol metabolism tested both by real-time RT-PCR and by microarray assay, with 10 of them having almost identical expression data obtained by either of the methods. In particular, phytosterol treatment was associated with significant increases in the expression of *Hmgcr* (2.2-fold;

Table 2
Real-time quantitative RT-PCR confirmation of changes in gene expression observed through microarray data analyses

Probe ID (Affymetrix)	Gene title	Gene symbol	Primer sequence	Fold change	
				Microarray analysis (*n*=3)	RT-PCR (*n*=6)
1449945_at	Peroxisome-proliferator-activated receptor, γ, coactivator 1 β	*Ppargc1b* (PGC-1β)	gaggagtcccttccctcatc tcctcgaaggttaaggctga	1.36	1.66 *
1459497_at	Farnesyl diphosphate farnesyl transferase 1	*Fdft1*	agcattgagccctttgaga caacatacgcagcaggctta	1.55	1.09
1417871_at	Hydroxysteroid (17-β) dehydrogenase 7	*Hsd17b7*	cctgtgctcagtccgttttt ccaaggccctgaattcaata	1.6	2.11 *
1459808_at	FK506-binding protein 4	*Fkbp4*	gtggttttccaagcctgtgt taccagagggcgttatcagg	1.45	1.02
1418288_at	Lipin 1	*Lpin1*	tgaaagccactgtcatctgc cctccaaaggtcacgagaga	3.03	3.67 *
1427229_at	3-Hydroxy-3-methylglutaryl-coenzyme A reductase	*Hmgcr*	tagcactggtccaggaaacc cacaggaacaaggcacacag	2.18	2.16 *
1440444_at	Fatty acid desaturase 1	*Fads1*	agggatgttggagtctggtg gagccataaaggcagcactc	1.88	1.54 *
1415993_at	Squalene epoxidase	*Sqle*	attggctcaggccttgtatg attgaaagcaacccaacagg	1.8	2.03 *
1422100_at	Cytochrome *P*450, family 7, subfamily a, polypeptide 1	*Cyp7a1*	gcatgtgtgagtgtgtgtgc gcctaagcattggactctgg	2.43	1.98
1421075_s_at	Cytochrome *P*450, family 7, subfamily b, polypeptide 1	*Cyp7B1*	caatttcccgttttccagag aatgcaaaggtaccctggtg	1.67	1.81 *
1419031_at	Fatty acid desaturase 2	*Fads2*	cacttaaagggtgcctcagc ccaaggacaaacacatgcag	1.6	2.17 *
1422533_at	Cytochrome *P*450, family 51	*Cyp51*	aacaacacagcagaggctga acagttcagtcgggaaaagg	1.79	1.8 *
1433445_x_at	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1	*Hmgcs1*	ggggagatggtgcatgtaat cggcaatggactctgagaa	1.61	1.79 *

* *P*<.05 as compared to controls.

P=.0002), *Hmgcs1* (1.8-fold; *P*=.001) and *Cyp7B1* (1.8-fold; *P*=.03). The expression levels of these genes tested by real-time RT-PCR were very similar to those observed by microarray data (Table 2 and Fig. 2).

Three candidate genes associated with adipogenesis and apoptosis modulation, namely, *FAS* [tumor necrosis factor (TNF) receptor superfamily member], *Id3* and *Hspa1a*, were selected for further confirmation by real-time RT-PCR techniques in all of the six samples from each experimental group (Fig. 3). Parallel to microarray assay data, real-time RT-PCR data showed significant alterations in the expression of *Hspa1a* (5.91-fold; *P*=.003) and *FAS* (0.65-fold; *P*=.003). The expression of *Id3* was not changed significantly (*P*=.22).

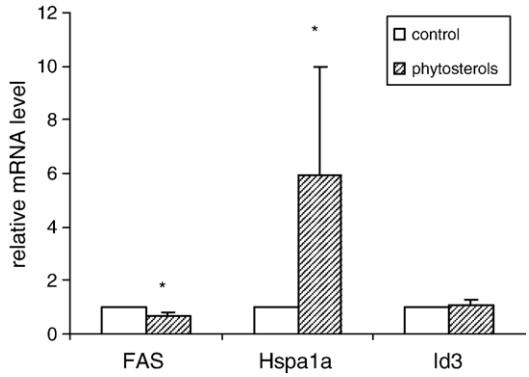

Fig. 3. Real-time RT-PCR analysis of the expression of selected hepatic genes related to adipogenesis and apoptosis modulation. Relative mRNA levels were obtained after normalization to β-actin (*n*=6). **P*<.05 as compared to controls.

4. Discussion

In the present study, we investigated the long-term effects of phytosterol treatment on hepatic gene expression in apo E-KO mice. We identified 132 genes/transcripts as differentially expressed between two groups, with an FDR of 0.23. Fifty seven of these genes were up-regulated, and the other 75 genes were down-regulated. The biological-pathway search analysis revealed that genes related to sterol metabolism, especially cholesterol synthesis, have been up-regulated by the long-term treatment of phytosterols. This result was further confirmed by real-time quantitative RT-PCR using a larger sample size (*n*=6). The genes involved in sterol metabolism included *Hmgcr*, *Hmgcs1*, *Hsd17b7*, *Sqle*, *Cyp7B1*, *Cyp51*, *Fads1*, *Fads2* and *Idi1*. In addition, the genes associated with adipogenesis (*FAS* and *Lpin1*) and apoptosis (*FAS* and *Hspa1a*) also showed altered expression. One interesting observation from this study is that all of these identified genes have been shown to be targets of sterol-regulatory element-binding proteins (SREBPs) — the transcription factors and key regulators of lipogenic genes in the liver [32–34]. The expression of PGC-1β, a coactivator of SREBPs, was also increased (Tables 1 and 2 and Fig. 2), suggesting its possible role in the modulation of SREBP levels with phytosterol treatment. However, the expression of SREBPs was not significantly altered.

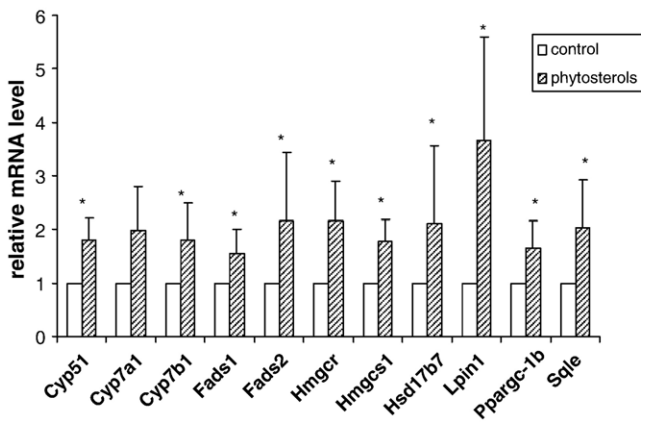

Fig. 2. Real-time RT-PCR analysis of the expression of hepatic genes related to sterol metabolism. Relative mRNA levels were obtained after normalization to β-actin (*n*=6). **P*<.05 as compared to controls.

Available evidence suggests that *Hmgcr* can be regulated both transcriptionally and posttranscriptionally in different animal models [35,36]. Our previous studies have shown that long-term treatment with phytosterols increased the activity of hepatic *Hmgcr* in the liver of apo E-KO mice [9]. In the present study, we further confirm an up-regulation in the expression of *Hmgcr* at the mRNA level. Thus, dietary phytosterols may not only increase enzyme activity but also increase its production as a feedback mechanism in response to blockage of intestinal cholesterol absorption. *mHsd17b7* (*17-HSD17b7*) efficiently catalyzes the conversion of inactive estrone to its biologically active hydroxy estradiol [37]. *mHsd17b7* is coexpressed with other cholesterogenic genes such as *Hmgcr* during murine embryonic development [38]. *Cyp51* is involved in the regulation of the first and rate-limiting step in the postsqualene portion of cholesterol biosynthesis through removal of the 14α-methyl group of lanosterol [39]. Activity of *Cyp51* is mainly regulated at the level of gene transcription, as cholesterol deprivation leads to an increase in mRNA levels and enzyme activity [40]. Squalene epoxidase (*Sqle*) catalyzes the conversion of squalene to 2,3-oxidosqualene [41] in sterol biosynthesis. *Sqle* activity has been shown to be regulated solely by changes in gene transcription [35,36], and dietary sterols may suppress its activity [42].

Dietary fat, including saturated and trans fatty acids, can induce the expression of PGC-1β (a transcriptional coactivator), along with SREBP1c/1a, in mice [43–45]. PGC-1β coactivates the SREBP and LXR families of transcription factors, thereby regulating de novo lipogenesis and lipoprotein secretion [45]. In agreement with these

observations, we observed a significant up-regulation in the expression of PGC-1β (1.66-fold; *P*=.007) in phytosterol-treated apo E-KO mice. It is possible that up-regulation of the PGC-1β gene induced by both dietary saturated and trans fats [45] and phytosterols is mediated through increases in cholesterol biosynthesis, as all of these dietary agents can induce cholesterol synthesis at least through different mechanisms.

Primary bile acids are formed from cholesterol in the liver mainly through the "classic pathway" or by an "alternative pathway" [46,47]. Cholesterol 7-α-hydroxylase (*Cyp7a1*) is the rate-limiting enzyme in the "classic" pathway of bile acid synthesis, while the "alternative" pathway is initiated by the sterol 27-hydroxylase (*Cyp27*), with subsequent 7β-hydroxylation of 27-hydroxycholesterol by the oxysterol 7β-hydroxylase (*Cyp7b1*) [48]. In this study, the expression of *Cyp7a1* was increased by 2.1-fold (*P*=.057), while the expression of *Cyp7b1* was increased by 1.8-fold (*P*=.025). Evidence suggests that the expression of cholesterol 7-hydroxylase is regulated by a number of stimuli, including bile acids, steroid hormones, thyroid hormones, cytokines, insulin, retinoids, dietary cholesterol, taurine, 2-aminoethanesulfonic acid and the digestion-resistant fraction of soybean [46,49–51]. We have observed an increase in the activity of *Cyp7a1* in our previous studies; however, the current study did not confirm a significant up-regulation in gene expression. This may suggest that posttranscriptional — rather than transcriptional — level is the most likely regulating factor under treatment with phytosterols in apo E-KO mice. The expression of *Cyp7b1* was up-regulated by 1.67-fold. However, previous studies have suggested that this up-regulation of *Cyp7b1* expression may not lead to increased bile acid synthesis and cholesterol catabolism [48]. As the LXR family plays a key role in lipid metabolism [52], one may expect dietary phytosterols to alter the expression of LXR target genes such as *ABCA1*, *ABCG1* and *ABCG8*. It should be mentioned that such alterations were not observed in the present study.

In conclusion, long-term treatment with dietary phytosterols results in significant reductions in plasma cholesterol levels and in the extent of atherosclerosis in apo E-KO mice. These changes are associated with alterations in the expression of several genes that regulate endogenous cholesterol synthesis and other steroid biosyntheses. Further investigations are needed to establish a relationship between the altered gene expression and marked antiatherogenic properties of dietary phytosterols, at least in apo E-KO mice.

Acknowledgments

This study was supported, in part, by the Natural Science and Engineering Research Council of Canada and the Heart and Stroke Foundation of Canada.

We thank Pingzhao Hu for his technical assistance with microarray data analysis. The authors are also very grateful to the Pathology Research Laboratory research team who participated in feeding animals and tissue collection, as well as other relevant activities.

References

[1] Peterson D. Effect of soybean sterols in the diet on plasma and liver cholesterol in chicks. Proc Soc Exp Biol Med 1951;78:143–7.
[2] Ostlund Jr RE. Phytosterols in human nutrition. Annu Rev Nutr 2002;22:533–49.
[3] Moghadasian MH. Pharmacological properties of plant sterols in vivo and in vitro observations. Life Sci 2000;67:605–15.
[4] Wolfs M, de Jong N, Ocke MC, Verhagen H, Monique Verschuren WM. Effectiveness of customary use of phytosterol/-stanol enriched margarines on blood cholesterol lowering. Food Chem Toxicol 2006; 44:1682–8.
[5] Lea LJ, Hepburn PA, Wolfreys AM, Baldrick P. Safety evaluation of phytosterol esters: Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides. Food Chem Toxicol 2004;42: 771–83.
[6] Nashed B, Yeganeh B, HayGlass KT, Moghadasian MH. Antiatherogenic effects of dietary plant sterols are associated with inhibition of proinflammatory cytokine production in Apo E-KO mice. J Nutr 2005; 135:2438–44.
[7] Moghadasian MH, McManus BM, Godin DV, Rodrigues B, Frohlich JJ. Proatherogenic and antiatherogenic effects of probucol and phytosterols in apolipoprotein E-deficient mice: possible mechanisms of action. Circulation 1999;99:1733–9.
[8] Yeganeh B, Moshtaghi-Kashanian GR, Declercq V, Moghadasian MH. Combination of dietary phytosterols plus niacin or fenofibrate: effects on lipid profile and atherosclerosis in apo E-KO mice. J Nutr Biochem 2005;16:222–8.
[9] Moghadasian MH, Nguyen LB, Shefer S, Salen G, Batta AK, Frohlich JJ. Hepatic cholesterol and bile acid synthesis, low-density lipoprotein receptor function, and plasma and fecal sterol levels in mice: effects of apolipoprotein E deficiency and probucol or phytosterol treatment. Metabolism 2001;50:708–14.
[10] Moghadasian MH. Dietary phytosterols reduce cyclosporine-induced hypercholesterolemia in apolipoprotein E-knockout mice. Transplantation 2006;81:207–13.
[11] Ikeda I, Tanabe Y, Sugano M. Effects of sitosterol and sitostanol on micellar solubility of cholesterol. J Nutr Sci Vitaminol (Tokyo) 1989; 35:361–9.
[12] von Bonsdorff-Nikander A, Christiansen L, Huikko L, Lampi AM, Piironen V, Yliruusi J, et al. A comparison of the effect of medium- vs. long-chain triglycerides on the in vitro solubilization of cholesterol and/or phytosterol into mixed micelles. Lipids 2005;40: 181–90.
[13] Plat J, Mensink RP. Increased intestinal ABCA1 expression contributes to the decrease in cholesterol absorption after plant stanol consumption. FASEB J 2002;16:1248–53.
[14] Lee MH, Lu K, Hazard S, Yu H, Shulenin S, Hidaka H, et al. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat Genet 2001;27:79–83.
[15] Hubacek JA, Berge KE, Cohen JC, Hobbs HH. Mutations in ATP-cassette binding proteins G5 (ABCG5) and G8 (ABCG8) causing sitosterolemia. Hum Mutat 2001;18:359–60.
[16] Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000;290:1771–5.
[17] Yu L, von Bergmann K, Lutjohann D, Hobbs HH, Cohen JC. Selective sterol accumulation in ABCG5/ABCG8-deficient mice. J Lipid Res 2004;45:301–7.
[18] Repa JJ, Dietschy JM, Turley SD. 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 2002;43:1864–74.

[19] Jolodar A, Hourihane S, Moghadasian M. Dietary phytosterols alter gene expression in the intestine of apolipoprotein E knockout mice. Nutr Res 2005;25:847–58.
[20] Moghadasian MH, McManus BM, Pritchard PH, Frohlich JJ. "Tall oil"-derived phytosterols reduce atherosclerosis in apo E-deficient mice. Arterioscler Thromb Vasc Biol 1997;17:119–26.
[21] Plat J, Kerckhoffs DA, Mensink RP. Therapeutic potential of plant sterols and stanols. Curr Opin Lipidol 2000;11:571–6.
[22] Quilez J, Rafecas M, Brufau G, Garcia-Lorda P, Megias I, Bullo M, et al. Bakery products enriched with phytosterol esters, alpha-tocopherol and beta-carotene decrease plasma LDL-cholesterol and maintain plasma beta-carotene concentrations in normocholesterolemic men and women. J Nutr 2003;133:3103–9.
[23] Moghadasian MH, Frohlich JJ. Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: clinical and experimental evidence. Am J Med 1999;107:588–94.
[24] Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 2003;31:1–8.
[25] Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 2004;5:R80.
[26] Affymetrix. Statistical algorithms description document. 2001 http://www.affymetrix.com/support/technical/whitepapers/sadd_whitepaper.pdf.
[27] Li C, Wong WH. DNA-Chip Analyzer (dChip) user manual. 2001 http://biosun1.harvard.edu/complab/dchip/filter_gene.htm.
[28] Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. PNAS 2001;98:5116–21.
[29] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene Ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000;25:25–9.
[30] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene Set Enrichment Analysis: a knowledge-based approach for interpreting genome-wide expression profiles. PNAS 2005;102:15545–50.
[31] Doniger SW, Salomonis N, Dahlquist KD, Vranizan K, Lawlor SC, Conklin BR. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. Genome Biol 2003;4:R7.
[32] Brown MS, Goldstein JL. Sterol regulatory element binding proteins (SREBPs): controllers of lipid synthesis and cellular uptake. Nutr Rev 1998;56:S1–S3.
[33] Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, et al. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. PNAS 2003;100:12027–32.
[34] Maxwell KN, Soccio RE, Duncan EM, Sehayek E, Breslow JL. Novel putative SREBP and LXR target genes identified by microarray analysis in liver of cholesterol-fed mice. J Lipid Res 2003;44:2109–19.
[35] Hidaka Y, Satoh T, Kamei T. Regulation of squalene epoxidase in HepG2 cells. J Lipid Res 1990;31:2087–94.
[36] Nakamura Y, Sakakibara J, Izumi T, Shibata A, Ono T. Transcriptional regulation of squalene epoxidase by sterols and inhibitors in HeLa cells. J Biol Chem 1996;271:8053–6.
[37] Nokelainen P, Peltoketo H, Vihko R, Vihko P. Expression cloning of a novel estrogenic mouse 17 beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase (m17HSD7), previously described as a prolactin receptor-associated protein (PRAP) in rat. Mol Endocrinol 1998;12:1048–59.
[38] Laubner D, Breitling R, Adamski J. Embryonic expression of cholesterogenic genes is restricted to distinct domains and colocalizes with apoptotic regions in mice. Brain Res Mol Brain Res 2003;115:87–92.
[39] Pikuleva IA. Cytochrome *P*450s and cholesterol homeostasis. Pharmacol Ther 2006;122:761–73.
[40] Stromstedt M, Rozman D, Waterman MR. The ubiquitously expressed human CYP51 encodes lanosterol 14 alpha-demethylase, a cytochrome *P*450 whose expression is regulated by oxysterols. Arch Biochem Biophys 1996;329:73–81.
[41] Chugh A, Ray A, Gupta JB. Squalene epoxidase as hypocholesterolemic drug target revisited. Prog Lipid Res 2003;42:37–50.
[42] Satoh T, Hidaka Y, Kamei T. Regulation of squalene epoxidase activity in rat liver. J Lipid Res 1990;31:2095–101.
[43] Lin J, Tarr PT, Yang R, Rhee J, Puigserver P, Newgard CB, et al. PGC-1beta in the regulation of hepatic glucose and energy metabolism. J Biol Chem 2003;278:30843–8.
[44] Meirhaeghe A, Crowley V, Lenaghan C, Lelliott C, Green K, Stewart A, et al. Characterization of the human, mouse and rat PGC1 beta (peroxisome-proliferator-activated receptor-gamma co-activator 1 beta) gene in vitro and in vivo. Biochem J 2003;373:155–65.
[45] Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, et al. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. Cell 2005;120:261–73.
[46] Myant NB, Mitropoulos KA. Cholesterol 7 alpha-hydroxylase. J Lipid Res 1977;18:135–53.
[47] Vlahcevic ZR, Pandak WM, Stravitz RT. Regulation of bile acid biosynthesis. Gastroenterol Clin North Am 1999;28:1–25.
[48] Pandak WM, Hylemon PB, Ren S, Marques D, Gil G, Redford K, et al. Regulation of oxysterol 7alpha-hydroxylase (CYP7B1) in primary cultures of rat hepatocytes. Hepatology 2002;35:1400–8.
[49] Russell DW, Setchell KD. Bile acid biosynthesis. Biochemistry 1992;31:4737–49.
[50] Chiang JY, Kimmel R, Stroup D. Regulation of cholesterol 7-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXR). Gene 2001;262:257–65.
[51] Han SH, Chung MJ, Lee SJ, Rhee C. Digestion-resistant fraction from soybean (*Glycine max* (L.) Merrill) induces hepatic LDL receptor and CYP7A1 expression in apolipoprotein E-deficient mice. J Nutr Biochem 2006;17:682–8.
[52] Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, et al. Role of LXRs in control of lipogenesis. Genes Dev 2000;14:2831–8.