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ORIGINAL ARTICLE

Activated polyamine catabolism leads to low cholesterol levels by enhancing bile acid synthesis

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Abstract Transgenic mice with activated polyamine catabolism due to overexpression of spermidine/spermine N^1-acetyltransferase (SSAT) have significantly reduced plasma total cholesterol levels. In our study, we show that low cholesterol levels were attributable to enhanced bile acid synthesis in combination with reduced cholesterol absorption. Hepatic cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme catalyzing the conversion of cholesterol to bile acids, plays an important role in the removal of excess cholesterol from the body. We suggest that by reducing activity of Akt activated polyamine catabolism increased the stability and activity of peroxisome proliferator-activated receptor γ co-activator 1α, the critical activator of CYP7A1. This is supported by our finding that the treatment with SSAT activator, N^1,N^{11}-diethylnorspermine, reduced significantly the amount of phosphorylated (active) Akt in HepG2 cells. In summary, activated-polyamine catabolism is a novel mechanism to regulate bile acid synthesis. Therefore, polyamine catabolism could be a potential therapeutic target to control hepatic CYP7A1 expression.

Keywords Spermidine/spermine N^1-acetyltransferase · Cholesterol · Bile acids · Cholesterol 7α-hydroxylase · Peroxisome proliferator-activated receptor γ co-activator 1α · Akt

Abbreviations

HMGCR	3-Hydroxy-3-methylglutaryl-CoA reductase
NPC1L1	Niemann–Pick C1-like 1 protein
CYP7A1	Cholesterol 7α-hydroxylase
PGC-1α	Peroxisome proliferator-activated receptor γ co-activator 1α
SSAT	Spermidine/spermine N^1-acetyltransferase
HDL	High-density lipoprotein
GLC	Gas–liquid chromatography
AMPK	5′-AMP-activated protein kinase
HNF-4α	Hepatocyte nuclear factor 4α
LXRα	Liver X receptor α
FXR	Farnesoid X receptor
SIRT1	Sirtuin 1
MAPK	Mitogen-activated protein kinase
PRMT1	Protein arginine methyltransferase 1
PI3K	Phosphatidylinositol 3-kinase
GSK-3β	Glycogen synthase kinase 3β
PPARδ	Peroxisome proliferator-activated receptor δ
DENSPM	N^1, N^{11}-diethylnorspermine
NTCP	Na^+-taurocholate cotransporting polypeptide
OATP1	Na^+-independent organic anion transporting polypeptide
BSEP	Bile salt export pump
FDFT1	Squalene synthase

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DHCR7	7-Dehydrocholesterol reductase
SREBP	Sterol regulatory element binding protein
ABCA1	ATP binding cassette protein A1
ACAT2	Acyl CoA: cholesterol acyltransferase 2
SR-BI	Class B type 1 scavenger receptor
ABCG5	ATP binding cassette protein G5
ABCG8	ATP binding cassette protein G8
PXR	Pregnane X receptor
CAR	Constitutive androstane receptor
PPARα	Peroxisome proliferator-activated receptor α
HDAC7	Histone deacetylase 7
ASBT	Apical Na^+-dependent bile acid transporter
I-BABP	Ileal bile acid binding protein
LDL	Low-density lipoprotein
VLDL	Very-low-density lipoprotein
IDL	Intermediate-density lipoprotein

Introduction

Hypercholesterolemia is one of the well-known causes for atherosclerosis and cardiovascular disease (Bhatnagar et al. 2008). Cholesterol homeostasis in mammals is maintained by a well-balanced control between supply and catabolism of cholesterol (Dietschy et al. 1993). Supply of cholesterol includes hepatic receptor-mediated uptake, hepatic de novo synthesis, and intestinal cholesterol absorption, the latter two of which are reciprocally regulated (Grundy et al. 1969). Hepatic conversion of cholesterol to bile acids is the principal catabolic pathway, and the only pathway whereby excess cholesterol is eliminated from the body (Russell and Setchell 1992). Therefore, bile acid synthesis is the critical step in the maintenance of cholesterol homeostasis.

Cholesterol lowering can be achieved in the body by reducing cholesterol synthesis, impairing intestinal cholesterol absorption or increasing elimination of cholesterol by enhancing bile acid synthesis. The formation of cholesterol is catalyzed by hepatic 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (Goldstein and Brown 1990) whereas the key intestinal cholesterol transporter protein is Niemann–Pick C1-like 1 protein (NPC1L1) (Altmann et al. 2004). The rate-limiting enzyme in the major (neutral or classic) bile acid synthetic pathway is cholesterol 7α-hydroxylase (CYP7A1) which promotes the formation of primary bile acids, cholic and chenodeoxycholic acid (Fuchs 2003; Russell and Setchell 1992). One of the critical activators of CYP7A1 transcription is peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) (Shin et al. 2003).

Polyamines, putrescine, spermidine and spermine, are positively charged aliphatic amines that exist in all eukaryotic cells (Jänne et al. 2005; Montanez et al. 2007). Spermidine/spermine N^1-acetyltransferase (SSAT) is the rate-controlling enzyme in the catabolism of polyamines, comprising the conversion of spermidine and spermine back to their precursor putrescine. Polyamines are essential for cell growth, but the role of polyamines in lipid metabolism is largely unknown.

In this study, we investigated the effects of activated polyamine catabolism on cholesterol metabolism in transgenic mice overexpressing SSAT. Here, we show that enhanced polyamine catabolism elicits a more active and stable PGC-1α protein in the liver causing the induction of hepatic CYP7A1 expression. Increased bile acid synthesis, bile excretion, and fecal loss of bile acids resulted in low circulating total cholesterol levels in SSAT mice. Therefore, enhanced polyamine catabolism is an important regulator of cholesterol homeostasis.

Materials and methods

Animals

The generation of transgenic (DBA/2 × Balb/c) mice overexpressing SSAT under endogenous SSAT promoter has been described (Pietilä et al. 1997). Mice used in experiments were 3- to 6-month-old females. Littermates of transgene mice were used as controls. The animals were housed on 12-h light/dark cycle at 22 ± 1°C and were fed a regular laboratory chow. The study protocols were approved by the Animal Care and Use Committee of the University of Kuopio and the Provincial Government.

Blood metabolites

Plasma or serum samples were taken from the saphenous vein before and/or after 12–13 h fasting. Plasma triglyceride, alkaline phosphatase, and alanine aminotransferase were determined using colorimetric or enzyme kinetic assay in Microlab 200 analyzer (Merck, Darmstadt, Germany). Serum high-density lipoprotein (HDL) cholesterol was analyzed by Hitachi 717 analyzer. Gas–liquid chromatography (GLC) method (Miettinen 1988) was used to measure total cholesterol, precursors of cholesterol synthesis (squalene, cholestenol, desmosterol and lathosterol), cholestanol (5α-saturated cholesterol derivative), and cholesterol absorption markers (campesterol and sitosterol) in plasma or liver samples. Squalene and non-cholesterol sterol values were expressed as the ratio of mmol/mol of cholesterol eliminating the variation in plasma cholesterol

levels. Serum total bile acid levels were analyzed as described in Miettinen and Koivisto (1983).

Fecal analyses

Pooled stool samples collected daily for 3–4 days were measured for the neutral sterols and bile acids with GLC (Miettinen 1982). Cholesterol synthesis was calculated as the difference between the sum of fecal sterols of cholesterol origin plus bile acids, and dietary cholesterol. Food intake was monitored for 4 days and cholesterol content of chow diet was analyzed with GLC.

Cholesterol and bile acid absorption was determined by the fecal ratio method (Wang and Carey 2003). Briefly, mice were orally gavaged with 1 μCi [^{14}C]cholesterol (GE Healthcare Bio-Sciences, Fairfield, CT, USA), 1 μCi [^{3}H]taurocholate (American Radiolabeled Chemicals Inc, St. Louis, MO, USA) and 2 μCi [^{3}H]sitostanol (American Radiolabeled Chemicals Inc, St. Louis, MO, USA) in 0.15 ml olive oil and feces were collected once a day for 4 days. Fecal samples were analyzed as described above.

Quantitative RT-PCR analyses

Total RNA from liver, jejunum, and ileum of was isolated using the acidic guanidinium thiocyanate method (Chomczynski and Sacchi 1987) or Trizol (Invitrogen Ltd, Carlsbad, CA, USA). DNase treatment, cDNA synthesis and quantitative RT-PCRs were performed as described previously (Pirinen et al. 2007). Data were normalized to expression of β-actin which expression was similar in SSAT and wild-type mice. Primer sets are available from the authors on request.

Western blot

Liver was homogenized in buffer containing 50 mM Tris–HCl pH 7.6, 0.15 M NaCl, 1 mM EDTA, 0.5% deoxycholic acid, 0.1% SDS, 1% Triton-X, 1 mM phenylmethylsulphonylfluoride in dimethyl sulfoxide, 10 mM Na_3VO_4, 100 mM NaF, 10 mM $Na_4P_2O_7$ and protease inhibitor cocktail (Roche Applied Science, Basel, Switzerland). A total of 10–25 μg of protein was boiled for 5 min, subjected to sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis, and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Membranes were blocked overnight with 5% nonfat dry milk or 2% ECL blocking reagent (GE Healthcare Bio-Sciences, Fairfield, CT, USA) in tris-buffered saline containing 0.1% Tween. Blots were probed with an antibody detecting the carboxyl terminus of PGC-1α (AB3242, Millipore, Billerica, MA, USA), hepatocyte nuclear factor 4α (HNF-4α) (sc-8997, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), liver X receptor α (LXRα) (ab28478, Abcam, UK), farnesoid X receptor (FXR) (sc-13063, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), phosphorylated α subunit of 5′-AMP-activated protein kinase (AMPK) (Thr172) (#2531, Cell Signaling Technology Inc, Danvers, MA, USA), sirtuin 1 (SIRT1) (07-131, Millipore, Billerica, MA, USA), dually phosphorylated p38 mitogen-activated protein kinase (MAPK) (Thr180/Tyr182) (#9215, Cell Signaling Technology Inc, Danvers, MA, USA), protein arginine methyltransferase 1 (PRMT1) (#2449, Cell Signaling Technology Inc, Danvers, MA, USA), phosphorylated Akt (Ser473) (#9271, Cell Signaling Technology Inc, Danvers, MA, USA), total Akt (#9272, Cell Signaling Technology Inc, Danvers, MA, USA), phosphatidylinositol 3-kinase (PI3K) (#4292, Cell Signaling Technology Inc, Danvers, MA, USA), phosphorylated glycogen synthase kinase 3β (GSK-3β) (Ser21/9) (#9331, Cell Signaling Technology Inc, Danvers, MA, USA), total GSK-3β (#9332, Cell Signaling Technology Inc, Danvers, MA, USA), peroxisome proliferator-activated receptor δ (PPARδ) (sc-7197, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, a kind gift from professor Carsten Carlberg's laboratory) or actin (sc-1616, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). This was followed by incubation with appropriate horseradish peroxidase-conjugated secondary antibody. Antibody-bound protein was detected using ECL plus western blotting detection reagent (GE Healthcare Bio-Sciences, Fairfield, CT, USA). Blots were visualized on the film or using a Typhoon scanner (GE Healthcare Bio-Sciences, Fairfield, CT, USA).

N^1,N^{11}-diethylnorspermine (DENSPM) treatment in HepG2 cells

The human hepatoma HepG2 cells were purchased from the American Type Culture Collection. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Hyclone, Thermo Scientific, Waltham, MA, USA) with 2 mM glutamine (Gibco, Invitrogen, Carlsbad, CA, USA) and 100 μg/ml streptomycin (Gibco, Carlsbad, CA, USA) and maintained at 37°C in 90% air and 10% carbon dioxide in 6-well plates. Cells were treated with or without 10 μM DENSPM for 48 h. SSAT activity was assayed as described in (Bernacki et al. 1995). Western blot analyzes was performed as previously described.

Statistical analysis

Statistical analysis was performed with Student's two-tailed t test. Data are expressed as mean ± SEM. $P < 0.05$ was considered as statistically significant.

Results

Variables of cholesterol metabolism in the fasting and fed state

In the fasting state, SSAT mice had significantly lower plasma total and HDL cholesterol than wild-type mice (Table 1). The plasma cholesterol precursors to cholesterol ratios, indicators of cholesterol synthesis, were all significantly higher, and of the cholesterol absorption markers, plasma cholestanol and campesterol to cholesterol ratios were lower in SSAT mice than in wild-type mice. In the fed state, liver total cholesterol pool was similar in SSAT mice as in wild-type mice (Table 1). Plasma total and HDL cholesterol levels remained lower in SSAT mice than in wild-type mice (Table 1). Of the cholesterol synthesis indicators, plasma lathosterol to cholesterol ratio remained significantly higher in SSAT mice than in wild-type mice, while the other indicators had a similar trend. Cholesterol absorption marker ratios tended to be lower in SSAT than in wild-type mice also in the fed state but the differences were not statistically significant. Serum total bile acid concentrations were significantly higher in SSAT than in wild-type mice in the fed state (Table 1).

Fecal data

Cholesterol absorption efficiency was significantly reduced in SSAT mice (Fig. 1a), a finding supported by the low plasma cholesterol absorption markers. In contrast, bile acid absorption efficiency was similar in SSAT mice as in wild-type mice (Fig. 1b) suggesting that increased serum bile acid levels were due to impaired hepatic bile acid absorption from the portal vein. In addition, fecal output of neutral sterols and bile acids was significantly increased in SSAT mice (Table 2). Only a small fraction of cholesterol was converted to its metabolites coprostanol and coprostanone (tg: 0.12 vs. wt: 0.08 mg/day, pooled data), and cholestanol (0.12 mg/day in both mice). Similarly, distribution of primary, secondary and tertiary bile acids was fairly similar in SSAT and wild-type mice (primary bile acids tg: 41 vs. wt: 26% of total bile acids, secondary and tertiary bile acids tg: 59 vs. wt: 73% of total bile acids). Due to the higher food consumption of SSAT mice (Jell et al. 2007; Pirinen et al. 2007), dietary intake of cholesterol was increased in SSAT mice (Table 2). Cholesterol synthesis was about threefold higher in SSAT mice than in wild-type mice (Table 2).

No liver or small intestine damage in SSAT mice

Alanine aminotransferase (tg: 39 ± 2 vs. wild-type: 38 ± 2 U/l, non-significant) and alkaline phosphatase (tg: 284 ± 24 vs. wild-type: 334 ± 21 U/l, non-significant) were not statistically different in SSAT and wild-type mice. In addition, histological analyses of liver and small intestine samples did not show any abnormalities in SSAT mice which is in agreement with previously published results (Pietilä et al. 1997; Pirinen et al. 2007).

Table 1 Variables of cholesterol metabolism in female SSAT and wild-type mice in the fasting and fed state

Variables	Fasting		Fed	
	Wild type	SSAT	Wild type	SSAT
Liver cholesterol (mg/100 g)	ND	ND	214 ± 4	206 ± 10
Plasma cholesterol, mmol/l[a]	2.2 ± 0.2	1.4 ± 0.1*	2.5 ± 0.1	1.7 ± 0.1***
Serum HDL cholesterol, mmol/l	1.7 ± 0.1	1.1 ± 0.3*	1.8 ± 0.3	1.1 ± 0.2**
Serum bile acids (total), μmol/l	ND	ND	3.8 ± 0.5	4.8 ± 0.3*
Plasma cholesterol precursors[b]				
Squalene	98 ± 10	188 ± 10***	133 ± 21	162 ± 12
Cholestenol	12 ± 2	28 ± 4**	14 ± 4	28 ± 9
Desmosterol	68 ± 9	156 ± 15***	61 ± 8	87 ± 12
Lathosterol	90 ± 10	149 ± 15**	75 ± 9	171 ± 26**
Plasma cholesterol absorption markers[b]				
Cholestanol	249 ± 17	169 ± 13**	263 ± 21	210 ± 21
Campesterol	1,414 ± 179	903 ± 106*	1,817 ± 218	1,284 ± 107
Sitosterol	410 ± 34	356 ± 20	495 ± 49	402 ± 25

Results are expressed as mean ± SEM (n = 5–6 mice per group)

ND not detected

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[a] Measured with GLC; to convert to mg/dl, multiply with 37.3

[b] $10^2 \times$ μmol/mmol of cholesterol

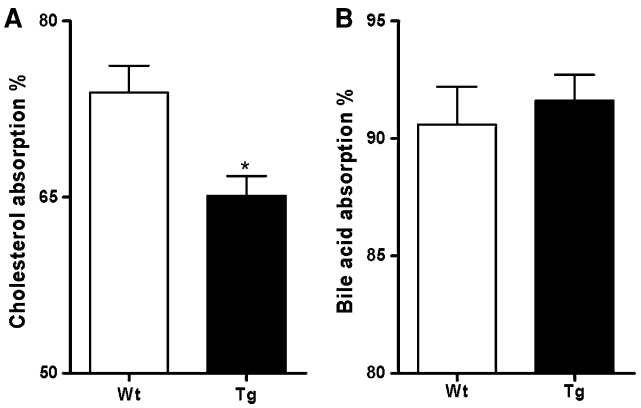


Fig. 1 SSAT mice have impaired cholesterol absorption but unaltered bile acid absorption in small intestine. **a** Cholesterol and **b** bile acid absorption efficiency were determined by triple fecal ratio method in SSAT and wild-type mice. Six-month-old female SSAT and wild-type mice were orally gavaged with 1 μCi [^{14}C]cholesterol; 1 μCi [^{3}H]taurocholate and 2 μCi [^{3}H]sitostanol and feces were collected once a day for 4 days. Results are presented as mean ± SEM of five to six mice per group. * $P < 0.05$

Table 2 Fecal neutral sterols, bile acids, dietary intake and cholesterol synthesis in female SSAT and wild-type mice

Variables	Wild type	SSAT
Total neutral sterols, mg/day	0.72 ± 0.07	1.81 ± 0.12***
Total bile acids, mg/day	1.5 1 ± 0.16	3.88 ± 0.41***
Dietary intake of cholesterol, mg/day	0.71 ± 0.04	1.42 ± 0.14***
Cholesterol synthesis, mg/day	1.51 ± 0.16	4.26 ± 0.27***

Results are expressed as mean ± SEM (n = 5–6 mice per group). Feces were collected in the fed state

*** $P < 0.001$

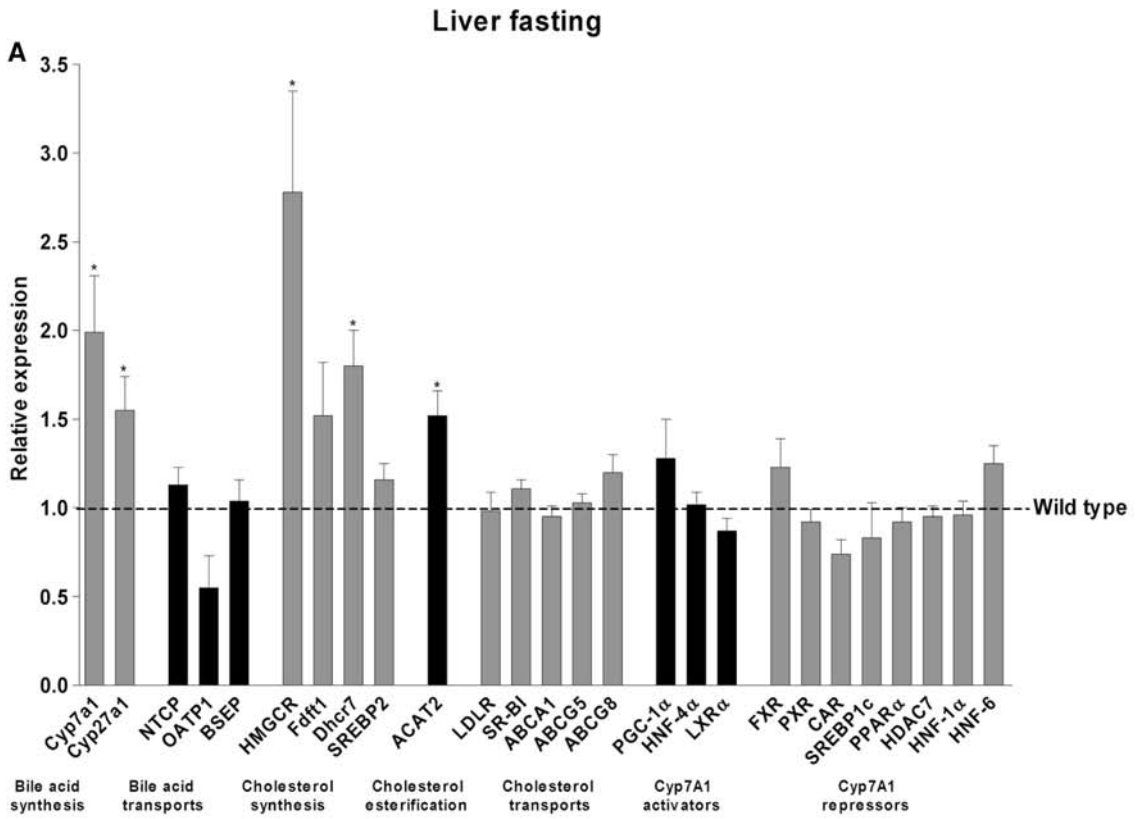
Increased bile acid synthesis in SSAT mice

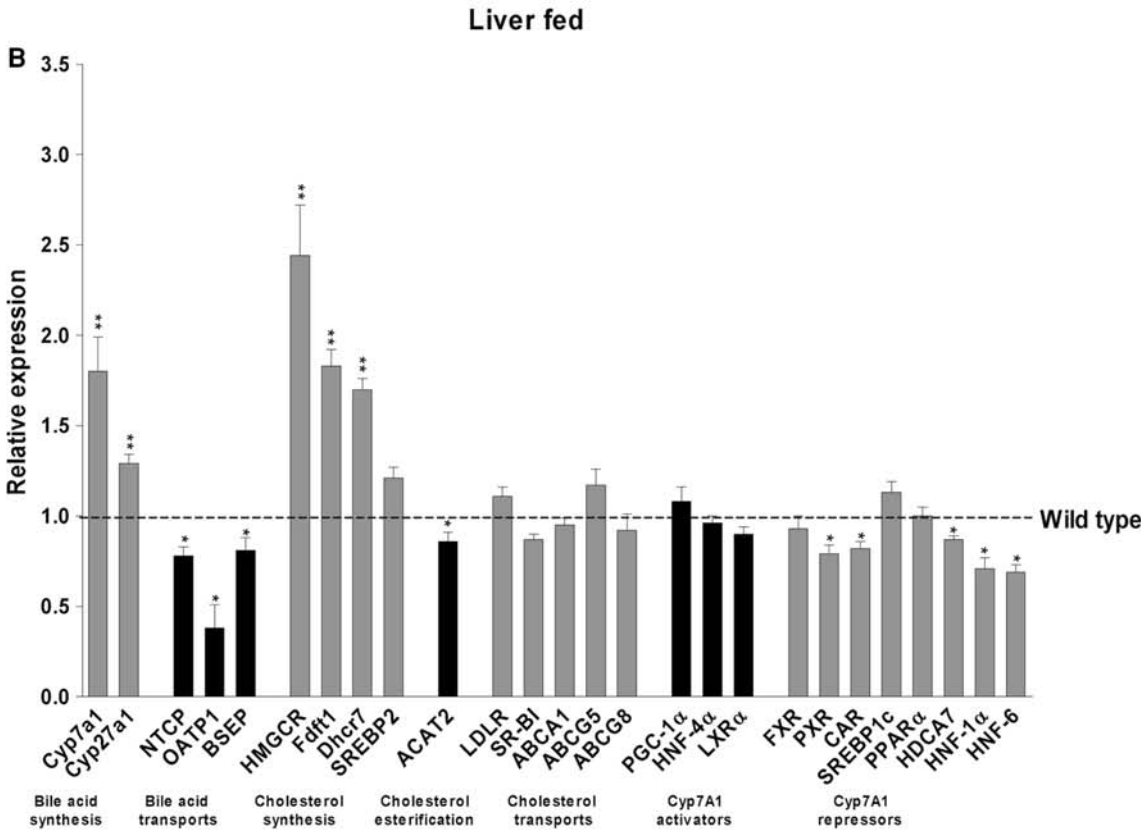
To investigate the molecular mechanisms leading to low cholesterol levels in SSAT mice, we analyzed expression of genes involved in cholesterol and bile acid metabolism in the liver and small intestine both in the fasting and fed state. CYP7A1 and sterol 27-hydroxylase, the rate-controlling genes for the neutral and acidic bile acid synthesis pathways, respectively, were upregulated in the livers of SSAT mice compared to wild-type mice in the fasting and fed state (Fig. 2a, b). Hepatic bile acid transporters, Na^+-taurocholate cotransporting polypeptide (NTCP) and Na^+-independent organic anion transporting polypeptide (OATP1), were significantly downregulated in the fed state. In the fasting state, expression of neither transporter significantly differed between SSAT and wild-type mice. Expression of bile salt export pump (BSEP), bile acid transporter exporting bile acids from hepatocytes to biliary canaliculi, was unchanged in the fasting state whereas expression was significantly reduced in the fed state.

Expression of genes related to biosynthesis of cholesterol [HMGCR, squalene synthase (FDFT1) and 7-dehydrocholesterol reductase (DHCR7)] was increased both in the fasting and fed state in SSAT mice. However, the increase in cholesterol biosynthesis was not mediated by sterol regulatory element binding protein (SREBP) 2 (SREBP2) since its expression was unchanged in SSAT mice (Fig. 2a, b). In SSAT mice, acyl CoA:cholesterol acyltransferase 2 (ACAT2), which esterifies cholesterol, was significantly induced in the fasting state, whereas in the fed state ACAT2 was downregulated. Expression of hepatic cholesterol transporters involved in influx [low-density lipoprotein receptor (LDLR) and class B type 1 scavenger receptor (SR-BI)] and efflux [ATP binding cassette protein A1 (ABCA1)] of cholesterol was similar in SSAT mice compared to wild-type mice. In addition, mRNA levels of biliary cholesterol transporters [ATP binding cassette protein G5 (ABCG5) and ATP binding cassette protein G8 (ABCG8)] were unchanged in both states.

CYP7A1 is mainly regulated at the gene transcriptional level by bile acids, cholesterol, amino acid taurine, cytokines, hormones, and transcription regulators (Fuchs 2003; Lam et al. 2006). To investigate why main neutral bile acid synthesis pathway was increased, hepatic expression of several factors activating or repressing CYP7A1 transcription was analyzed in the fasting and fed state (Fig. 2a, b). One of the key activators of CYP7A1 transcription is PGC-1α (Shin et al. 2003). PGC-1α activates CYP7A1 transcription together with HNF-4α by increasing HNF-4α-mediated transactivation of CYP7A1 (De Fabiani et al. 2003). The association of PGC-1α with HNF-4α or the binding of HNF-4α to the CYP7A1 promoter is interfered by pregnane X receptor (PXR) (Bhalla et al. 2004), constitutive androstane receptor (CAR) (Miao et al. 2006), SREBP1c (Ponugoti et al. 2007) and peroxisome proliferator-activated receptor α (PPARα) (Cheema and Agellon 2000). Another essential transcriptional activator of CYP7A1 is LXRα which mediates the stimulation of CYP7A1 transcription by dietary cholesterol in rodents but not in humans (Chiang et al. 2001). Repression of CYP7A1 transcription by bile acids is mediated through various mechanisms in which FXR (Holt et al. 2003; Lu et al. 2000; Makishima et al. 1999), histone deacetylase 7 (HDAC7) (Mitro et al. 2007), HNF-1α (Jung and Kullak-Ublick 2003) and HNF-6 (Wang et al. 2004) play important roles. However, our results showed that none of the essential activators of CYP7A1 transcription was upregulated. Of the repressors, expression of PXR, CAR, HDAC7, HNF-1α and HNF-6 was significantly lower in SSAT mice than in wild-type mice but only in the fed state. In addition, the reduction in the expression of these factors was minor (21, 18, 13, 29, and 31%, respectively).

In the jejunum, the main cholesterol transporter NPC1L1 was significantly decreased both in the fasting and


Liver fasting
A
Relative expression
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Wild type
Cyp7a1
Cyp27a1
NTCP
OATP1
BSEP
HMGCR
Fdft1
Dhcr7
SREBP2
ACAT2
LDLR
SR-BI
ABCA1
ABCG5
ABCG8
PGC-1α
HNF-4α
LXRα
FXR
PXR
CAR
SREBP1c
PPARα
HDAC7
HNF-1α
HNF-6
Bile acid synthesis
Bile acid transports
Cholesterol synthesis
Cholesterol esterification
Cholesterol transports
Cyp7A1 activators
Cyp7A1 repressors


Liver fed
B
Relative expression
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Wild type
Cyp7a1
Cyp27a1
NTCP
OATP1
BSEP
HMGCR
Fdft1
Dhcr7
SREBP2
ACAT2
LDLR
SR-BI
ABCA1
ABCG5
ABCG8
PGC-1α
HNF-4α
LXRα
FXR
PXR
CAR
SREBP1c
PPARα
HDCA7
HNF-1α
HNF-6
Bile acid synthesis
Bile acid transports
Cholesterol synthesis
Cholesterol esterification
Cholesterol transports
Cyp7A1 activators
Cyp7A1 repressors

◀ **Fig. 2** Hepatic gene expression levels of SSAT mice in **a** the fasting and **b** fed state compared to wild-type mice. Data are mean ± SEM of five to eight mice per group. The expression of selected genes was analyzed by quantitative RT-PCR from 4- to 6-month-old female mice. * $P < 0.05$ and ** $P < 0.01$. Beta-actin was used as a normalization gene. CYP27A1, sterol 27-hydroxylase

fed state (Fig. 3a, b). However, expression of the regulator of NPC1L1 transcription, PPARδ (van der Veen et al. 2005), was similar in SSAT and wild-type mice in the fasting and fed state (Fig. 3a, b). SR-BI, the major protein responsible for the cholesterol binding in intestinal brush border membrane vesicle, did not differ between mouse lines in the fasting state whereas expression was significantly reduced in the fed state. In addition, expression of cholesterol transporters involved in the transfer of cholesterol from enterocytes to lumen (ABCA1, ABCG5, and ABCG8) was similar in SSAT and wild-type mice in the fasting and fed state. In the fasting state, statistically significant changes were neither observed in expression of ACAT2, which esterifies cholesterol taken up by enterocytes, nor observed in PPARα, which regulates expression of SR-BI. In contrast, in the fed state both genes were significantly downregulated in SSAT mice.

In ileum, no change in expression level of apical Na^+-dependent bile acid transporter (ASBT) or ileal bile acid binding protein (I-BABP) was observed between the mouse lines either in the fasting or in the fed state (Fig. 3c, d).

Increased PGC-1α protein levels explain the induction of CYP7A1 in the livers of SSAT mice

As gene expression analyses did not reveal a clear reason for the induction of CYP7A1, we analyzed protein levels of the key regulators of CYP7A1 (FXR, HNF-4α, LXRα, and PGC-1α). The protein amounts of FXR, HNF-4α, and LXRα were unchanged but PGC-1α protein levels were about doubled in the livers of SSAT mice in the fed state (Fig. 4) despite of unchanged mRNA levels (Fig. 2a, b). In contrast, there was no significant increase in PGC-1α mRNA and protein levels in jejunum in SSAT and wild-type mice (data not shown). Because enhanced polyamine catabolism induces AMPK in WAT of SSAT mice by depleting ATP pool (Pirinen et al. 2007), we hypothesized that the hepatic elevation of PGC-1α protein levels could be as well caused by the activation of AMPK that phosphorylates PGC-1α at Thr177 and Ser538 producing a more stable protein (Jager et al. 2007). However, western blots revealed that AMPK was not induced in the livers of SSAT mice in the fed state (Fig. 4). In addition to phosphorylation by AMPK, the more stable and/or active PGC-1α protein is achieved through deacetylation by SIRT1

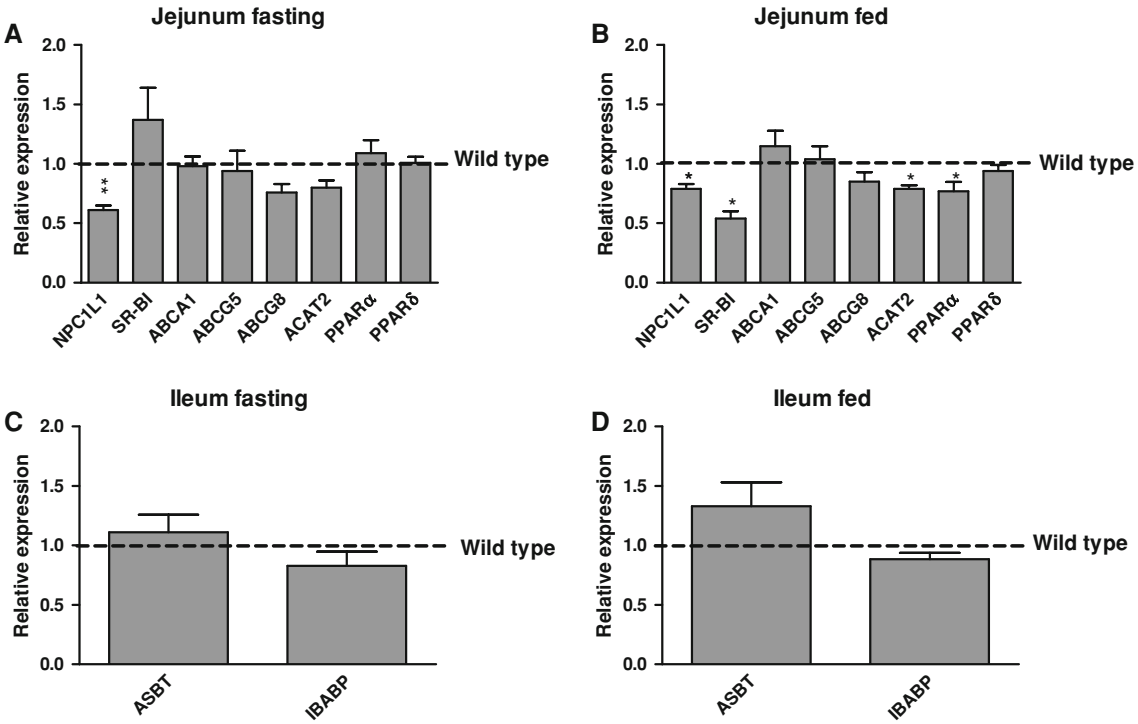


Fig. 3 Quantitative RT-PCR analysis for intestinal mRNA levels in SSAT and wild-type mice. Selected gene expression profiles in jejunum (**a**, **b**) and ileum (**c**, **d**) in the fasting and fed state; respectively. Results are presented as mean ± SEM of 6–11 mice per group. * $P < 0.05$ and ** $P < 0.01$. Mice used for the analysis were 4- to 6-month-old females

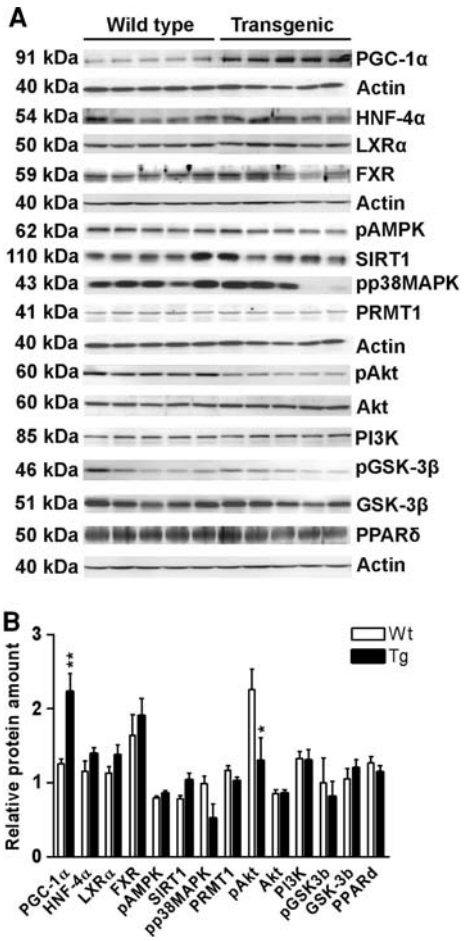


Fig. 4 Protein levels of PGC-1α and its regulators in the livers of SSAT and wild-type mice in the fed state. **a** PGC-1α, HNF-4α, LXRα, FXR, phosphorylated AMPK (*pAMPK*), SIRT1, phosphorylated p38MAPK (*pp38 MAPK*), PRMT1, phosphorylated Akt (pAkt), total Akt, PI3 K, phosphorylated GSK-3β (*pGSK-3β*), GSK-3β, PPARδ and actin protein levels in the livers from 6-month-old female SSAT and wild-type mice. See "Materials and methods" for details. **b** Relative protein levels after normalization to actin. Data are mean ± SEM of five mice per group. * $P < 0.05$ and ** $P < 0.01$

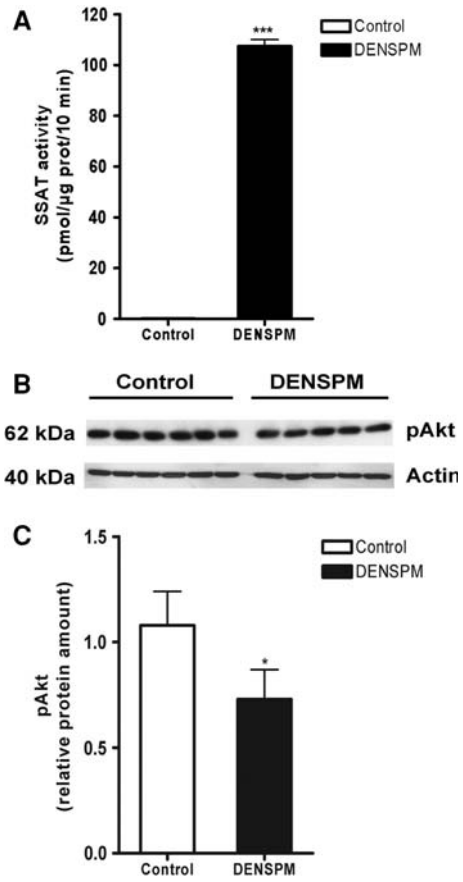


Fig. 5 Effect of activated polyamine catabolism on phosphorylated Akt levels in HepG2 cells. The cells were grown with or without 10 μM DENSPM for 48 h. **a** SSAT activity with (*black bar*) and without (*white bars*) DENSPM treatment. Results are mean ± SEM of nine cultures per group. *** $P < 0.001$. **b** Western blot analysis showing the effect of DENSPM treatment on the protein levels of phosphorylated Akt (*pAkt*) and actin. See "Materials and methods" for details. **c** Relative protein amount of phosphorylated Akt in control (*white bar*) or DENSPM-treated (*black bar*) HepG2 cells. Results are presented as mean ± SEM of five to eight culture per group. * $P < 0.05$

(Rodgers et al. 2005), phosphorylation by p38MAPK (Puigserver et al. 2001), inhibition of PGC-1α phosphorylation by GSK-3β (Olson et al. 2008) or methylation by PRMT1 (Teyssier et al. 2005). Furthermore, PPARδ increases PGC-1α protein levels in the absence of increase in PGC-1α mRNA levels (Hancock et al. 2008). Therefore, protein levels of these regulators were analyzed. However, protein levels of PRMT1, SIRT1, phosphorylated, i.e., active (Han et al. 1994), p38MAPK, phosphorylated and total GSK-3β and PPARδ were unaltered in the fed state (Fig. 4). The induction of PGC-1α was best explained when western blots of an inhibitor of PGC-1α (Li et al. 2007), Akt, were analyzed. The level of phosphorylated, i.e., active (Alessi et al. 1996), Akt was reduced by ~ 45% in the livers of SSAT mice fed state (Fig. 4). Therefore, more stable PGC-1α protein with higher activity may be obtained when the PGC-1α phosphorylation by Akt is reduced (Rodgers et al. 2008). Akt is a direct downstream target of PI3K and as PI3K protein levels were unaltered in the livers of SSAT mice (Fig. 4), the reduction in phosphorylated Akt levels seems to be mediated via PI3K-independent mechanism.

DENSPM treatment in HepG2 cells

To test whether activated polyamine catabolism can reduce the protein amount of phosphorylated Akt in liver cells similarly as in glioblastoma (Jiang et al. 2007) and breast cancer cells (Nair et al. 2007), HepG2 cells were treated with or without a SSAT-inducing polyamine analog

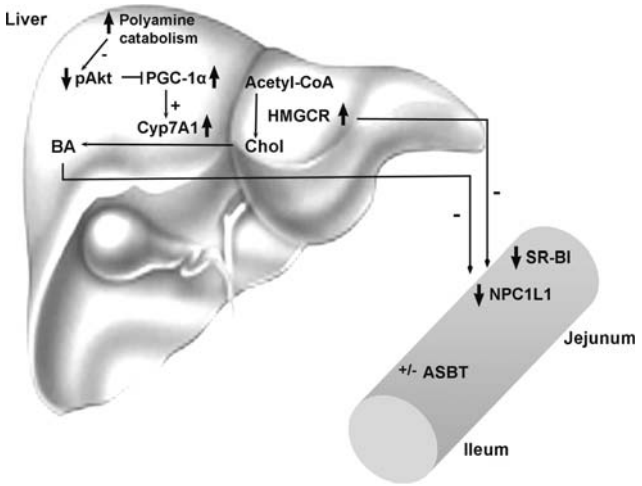


Fig. 6 The possible mechanism causing low cholesterol levels in SSAT mice. We hypothesize that in the livers of SSAT mice, the reduction in the levels of phosphorylated Akt (pAkt) caused by enhanced polyamine catabolism leads to the reduced phosphorylation of PGC-1α by Akt. This results in a more stabile and active of PGC-1α protein which is in turn able to induce CYP7A1, the rate-limiting gene of the main bile acid synthesis pathway. Enhanced conversion of cholesterol to bile acids increases compensatorily the rate of cholesterol synthesis which in turn lowers intestinal cholesterol absorption. In addition, it has been shown that increased hepatic bile acid pool size decreases the expression of NPC1L1 in jejunum (Ratliff et al. 2006). *BA* bile acid and *chol* cholesterol

DENSPM. DENSPM treatment induced dramatically SSAT activity in HepG2 cells at 48 h (Fig. 5a). In parallel, a significant reduction in the phosphorylation of Akt was noticed 48 h after the addition of DENSPM to the cells (Fig. 5b, c). Therefore, activated polyamine catabolism can decrease Akt phosphorylation also in vitro in liver cells.

Discussion

The novel finding in our study was that the activation of polyamine catabolism resulted in lowered plasma total cholesterol level by shunting cholesterol into bile acid synthesis leading to the elimination of cholesterol from the body. Enhanced bile acid synthesis was attributable to an increase in the activity and stability of the critical activator of bile acid synthesis, PGC-1α. Therefore, activated polyamine catabolism profoundly affects metabolism of cholesterol and bile acids as summarized in Fig. 6.

SSAT mice had significantly lower plasma total cholesterol levels than wild-type mice in the fasting and fed state. Low cholesterol level can result from reduction in cholesterol synthesis or absorption, or increase in cholesterol elimination. Cholesterol synthesis was increased but cholesterol absorption decreased in SSAT mice (Fig. 6). It is possible that reduced cholesterol absorption could lead to the induction of hepatic de novo cholesterol synthesis and subsequent increase in hepatic cholesterol pool which stimulates CYP7A1 transcription via activation of LXRα (Russell 1999). Since the mRNA and protein levels of LXRα were unchanged in SSAT mice, the primary cause for low cholesterol levels in SSAT mice was most likely CYP7A1-mediated enhanced conversion of cholesterol into bile acids (Fig. 6). Consequently, cholesterol synthesis was compensatorily increased to maintain hepatic cholesterol pool size unchanged. The enhancement of hepatic de novo cholesterol synthesis in turn reduced intestinal cholesterol absorption due to reciprocal regulation of these pathways. Another possibility is that CYP7A1-caused expansion of the hepatic bile acid pool resulted in the reduction in expression of NPC1L1 and thus reduced cholesterol absorption (Ratliff et al. 2006).

The decrease in total circulating cholesterol levels in SSAT mice was due to the lowering of low-density lipoprotein (LDL)/very low-density lipoprotein (VLDL)/intermediate-density lipoprotein (IDL) and HDL fractions. Given that the hepatic gene expression of two major cholesterol uptake receptors, SR-BI and LDLR, were unaltered, it is unlikely that increased hepatic cholesterol clearance from the circulation was responsible for reduced LDL and HDL cholesterol levels in SSAT mice. Because SSAT mice had increased bile acid synthesis, newly synthesized cholesterol is likely to be shunted to the formation of bile acids reducing the availability of cholesterol for VLDL and HDL synthesis. Consistent with this hypothesis, ACAT2 expression was significantly reduced in the fed state. Reduction of ACAT2 activity diminishes the assembly and secretion of apolipoprotein B-containing lipoproteins such as VLDL (Leon et al. 2005). Moreover, the expression of the key regulator of HDL formation, ABCA1, did not differ between SSAT and wild-type mice. However, the detailed mechanisms responsible for reduced LDL/VLDL/IDL and HDL cholesterol fractions in SSAT mice remain to be determined.

SSAT mice had significantly elevated serum total bile acid levels. Correspondingly, the expression of bile acid uptake transporters, NTCP and OATP1, was reduced in the liver. This may act as a mechanism to prevent accumulation of toxic levels of bile acids in the liver. Furthermore, SSAT mice exhibited increased biliary bile acid excretion since fecal bile acid levels were more than doubled. However, mRNA level of the main hepatic bile acid exporter, BSEP, was not increased but it is known that BSEP is also regulated by posttranscriptional mechanisms (Stieger et al. 2007). Because ileal bile acid absorption was unchanged in SSAT mice, increased bile acid excretion most likely leads to prolonged exposure of intestine to elevated bile acid concentrations which is known to predispose to carcinogenesis (Reddy et al. 1977). Indeed,

SSAT overexpression has been shown to enhance tumorigenesis in mice susceptible to intestinal cancer, $APC^{MIN/+}$ mice (Debruyne et al. 2001; Tucker et al. 2005).

Our conclusion that the low cholesterol levels in SSAT mice were attributable to the CYP7A1-mediated mechanism is supported by previous studies. Transient adenoviral overexpression of CYP7A1 in hamsters (Spady et al. 1995) and stable germ line transmission of CYP7A1 overexpression in mice (Miyake et al. 2001) lowered total cholesterol levels and compensatorily increased de novo hepatic cholesterol synthesis. Moreover, mice overexpressing CYP7A1 have reduced intestinal cholesterol absorption due to downregulation of NPC1L1 (Ratliff et al. 2006). Because overexpression of CYP7A1 prevents the development of diet-induced hypercholesterolemia (Ratliff et al. 2006) and atherosclerosis (Miyake et al. 2002) in mice, it has been suggested that CYP7A1 is an attractive therapeutic target for hypercholesterolemia. Indeed, our study supports this view. Therefore, the identification of new inducers of bile acid synthesis may offer novel strategies for drug development for hypercholesterolemia.

The molecular mechanism for the induction of hepatic CYP7A1 in SSAT mice is likely to be a consequence of increased stability and activity of PGC-1α (Fig. 6). PGC-1α is the transcriptional coactivator regulating mainly energy metabolism (Liang and Ward 2006) but it has also been shown to induce bile acid synthesis via the activation of Cyp7A1 (Shin et al. 2003). The transcription of PGC-1α is induced in the liver, for instance, by cAMP response element binding protein (Herzig et al. 2001), estrogen-related receptor α (Laganiere et al. 2004) and PPARδ (Hondares et al. 2007), mRNA levels of which were not increased in the livers of SSAT mice (data not shown). Moreover, the activity or stability of PGC-1α is also post-translationally regulated through phosphorylation by AMPK (Jager et al. 2007), p38MAPK (Puigserver et al. 2001), GSK-3β (Olson et al. 2008) and Akt (Li et al. 2007), deacetylation by SIRT1 (Rodgers et al. 2005) and methylation by PRMT1 (Teyssier et al. 2005). Because mRNA levels of PGC-1α were not elevated in the livers of SSAT mice, we conclude that the reason for elevated PGC-1α protein levels is due to protein modification leading to stabilization and increased activity of PGC-1α. Our western blot analyses showed that the most probable reason for increased PGC-1α protein levels was decreased phosphorylation of Akt resulting in a less active Akt (Fig. 6). When the activity of Akt is lowered, phosphorylation of PGC-1α by Akt is decreased supposedly leading to more stable and active PGC-1α (Li et al. 2007; Rodgers et al. 2008). Because DENSPM, a SSAT activator, has been shown to reduce phosphorylation of Akt in glioblastoma (Jiang et al. 2007) and breast cancer cells (Nair et al. 2007), activated polyamine catabolism may cause similar phenomenon in the liver cells. Indeed, this notion was supported by our findings that DENSPM treatment significantly downregulated the protein amount of phosphorylated Akt in HepG2 cells. The reduction in phosphorylated Akt levels in SSAT mice is likely to occur via PI3K-independent pathway, as reported previously in DENSPM-treated glioblastoma cells (Jiang et al. 2007), since PI3K protein levels were unaltered in the livers of SSAT mice. The mechanism via which activated polyamine catabolism regulates the phosphorylation of Akt in liver cells needs further investigation.

In summary, we demonstrated that SSAT mice have markedly reduced plasma total cholesterol levels due to enhanced hepatic expression of CYP7A1. This in turn leads to increased conversion of cholesterol into bile acids and results in reduced cholesterol absorption. The activation of CYP7A1 was a consequence of stabilization and activation of PGC-1α which was elicited by activated polyamine catabolism-induced reduction in the activity of Akt. To achieve the beneficial changes in cholesterol homeostasis, fairly low SSAT activity is needed for the activation of PGC-1α in the liver. Therefore, the activation of polyamine catabolism could be a potential target in the development of drugs which lower cholesterol levels.

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References

Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J 15:6541–6551

Altmann SW, Davis HR Jr, Zhu LJ, Yao X, Hoos LM, Tetzloff G, Iyer SP, Maguire M, Golovko A, Zeng M, Wang L, Murgolo N, Graziano MP (2004) Niemann–Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science 303:1201–1204

Bernacki RJ, Oberman EJ, Seweryniak KE, Atwood A, Bergeron RJ, Porter CW (1995) Preclinical antitumor efficacy of the polyamine analogue N1,N11-diethylnorspermine administered by multiple injection or continuous infusion. Clin Cancer Res 1:847–857

Bhalla S, Ozalp C, Fang S, Xiang L, Kemper JK (2004) Ligand-activated pregnane X receptor interferes with HNF-4 signaling by targeting a common coactivator PGC-1alpha. Functional implications in hepatic cholesterol and glucose metabolism. J Biol Chem 279:45139–45147

Bhatnagar D, Soran H, Durrington PN (2008) Hypercholesterolaemia and its management. BMJ 337:a993

Cheema SK, Agellon LB (2000) The murine and human cholesterol 7alpha-hydroxylase gene promoters are differentially responsive to regulation by fatty acids mediated via peroxisome proliferator-activated receptor alpha. J Biol Chem 275:12530–12536

Chiang JY, Kimmel R, Stroup D (2001) Regulation of cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRalpha). Gene 262:257–265

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. Anal Biochem 167:156–159

De Fabiani E, Mitro N, Gilardi F, Caruso D, Galli G, Crestani M (2003) Coordinated control of cholesterol catabolism to bile acids and of gluconeogenesis via a novel mechanism of transcription regulation linked to the fasted-to-fed cycle. J Biol Chem 278:39124–39132

Debruyne PR, Bruyneel EA, Li X, Zimber A, Gespach C, Mareel MM (2001) The role of bile acids in carcinogenesis. Mutat Res 480–481:359–369

Dietschy JM, Turley SD, Spady DK (1993) Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res 34:1637–1659

Fuchs M (2003) Bile acid regulation of hepatic physiology: III. Regulation of bile acid synthesis: past progress and future challenges. Am J Physiol Gastrointest Liver Physiol 284:G551–G557

Goldstein JL, Brown MS (1990) Regulation of the mevalonate pathway. Nature 343(6257):425–430

Grundy SM, Ahrens EH Jr, Davignon J (1969) The interaction of cholesterol absorption and cholesterol synthesis in man. J Lipid Res 10:304–315

Han J, Lee JD, Bibbs L, Ulevitch RJ (1994) A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science 265:808–811

Hancock CR, Han DH, Chen M, Terada S, Yasuda T, Wright DC, Holloszy JO (2008) High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proc Natl Acad Sci USA 105:7815–7820

Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 413:179–183

Holt JA, Luo G, Billin AN, Bisi J, McNeill YY, Kozarsky KF, Donahee M, Wang DY, Mansfield TA, Kliewer SA, Goodwin B, Jones SA (2003) Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. Genes Dev 17:1581–1591

Hondares E, Pineda-Torra I, Iglesias R, Staels B, Villarroya F, Giralt M (2007) PPARdelta, but not PPARalpha, activates PGC-1alpha gene transcription in muscle. Biochem Biophys Res Commun 354:1021–1027

Jager S, Handschin C, St-Pierre J, Spiegelman BM (2007) AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci USA 104:12017–12022

Jänne J, Alhonen L, Keinänen TA, Pietilä M, Uimari A, Pirinen E, Hyvönen MT, Järvinen A (2005) Animal disease models generated by genetic engineering of polyamine metabolism. J Cell Mol Med 9:865–882

Jell J, Merali S, Hensen ML, Mazurchuk R, Spernyak JA, Diegelman P, Kisiel ND, Barrero C, Deeb KK, Alhonen L, Patel MS, Porter CW (2007) Genetically altered expression of spermidine/spermine N^1-acetyltransferase affects fat metabolism in mice via acetyl-CoA. J Biol Chem 282:8404–8413

Jiang R, Choi W, Hu L, Gerner EW, Hamilton SR, Zhang W (2007) Activation of polyamine catabolism by N^1,N^{11}-diethylnorspermine alters the cellular localization of mTOR and downregulates mTOR protein level in glioblastoma cells. Cancer Biol Ther 6:1644–1648

Jung D, Kullak-Ublick GA (2003) Hepatocyte nuclear factor 1 alpha: a key mediator of the effect of bile acids on gene expression. Hepatology 37:622–631

Laganiere J, Tremblay GB, Dufour CR, Giroux S, Rousseau F, Giguere V (2004) A polymorphic autoregulatory hormone response element in the human estrogen-related receptor alpha (ERRalpha) promoter dictates peroxisome proliferator-activated receptor gamma coactivator-1alpha control of ERRalpha expression. J Biol Chem 279:18504–18510

Lam NV, Chen W, Suruga K, Nishimura N, Goda T, Yokogoshi H (2006) Enhancing effect of taurine on CYP7A1 mRNA expression in HepG2 cells. Amino Acids 30:43–48

Leon C, Hill JS, Wasan KM (2005) Potential role of acyl-coenzyme A:cholesterol transferase (ACAT) inhibitors as hypolipidemic and antiatherosclerosis drugs. Pharm Res 22:1578–1588

Li X, Monks B, Ge Q, Birnbaum MJ (2007) Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator. Nature 447:1012–1016

Liang H, Ward WF (2006) PGC-1alpha: a key regulator of energy metabolism. Adv Physiol Educ 30:145–151

Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ (2000) Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol Cell 6:507–515

Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B (1999) Identification of a nuclear receptor for bile acids. Science 284:1362–1365

Miao J, Fang S, Bae Y, Kemper JK (2006) Functional inhibitory cross-talk between constitutive androstane receptor and hepatic nuclear factor-4 in hepatic lipid/glucose metabolism is mediated by competition for binding to the DR1 motif and to the common coactivators, GRIP-1 and PGC-1alpha. J Biol Chem 281:14537–14546

Miettinen TA (1982) Gas–liquid chromatographic determination of fecal neutral sterols using a capillary column. Clin Chim Acta 124:245–248

Miettinen TA (1988) Cholesterol metabolism during ketoconazole treatment in man. J Lipid Res 29:43–51

Miettinen TA, Koivisto P (1983) Non-cholesterol sterols and bile acid production in hypercholesterolaemic patients with ileal bypass. In: Paumgartner GA, Stiehl AW, Gerok W (eds) Bile acids and cholesterol in health and disease. MTP press, Lancaster, pp 183–187

Mitro N, Godio C, De Fabiani E, Scotti E, Galmozzi A, Gilardi F, Caruso D, Vigil Chacon AB, Crestani M (2007) Insights in the regulation of cholesterol 7alpha-hydroxylase gene reveal a target for modulating bile acid synthesis. Hepatology 46:885–897

Miyake JH, Doung XD, Strauss W, Moore GL, Castellani LW, Curtiss LK, Taylor JM, Davis RA (2001) Increased production of apolipoprotein B-containing lipoproteins in the absence of hyperlipidemia in transgenic mice expressing cholesterol 7alpha-hydroxylase. J Biol Chem 276:23304–23311

Miyake JH, Duong-Polk XT, Taylor JM, Du EZ, Castellani LW, Lusis AJ, Davis RA (2002) Transgenic expression of cholesterol-7-alpha-hydroxylase prevents atherosclerosis in C57BL/6 J mice. Arterioscler Thromb Vasc Biol 22:121–126

Montanez R, Sanchez-Jimenez F, Aldana-Montes JF, Medina MA (2007) Polyamines: metabolism to systems biology and beyond. Amino Acids 33:283–289

Nair SK, Verma A, Thomas TJ, Chou TC, Gallo MA, Shirahata A, Thomas T (2007) Synergistic apoptosis of MCF-7 breast cancer cells by 2-methoxyestradiol and bis(ethyl)norspermine. Cancer Lett 250:311–322

Olson BL, Hock MB, Ekholm-Reed S, Wohlschlegel JA, Dev KK, Kralli A, Reed SI (2008) SCF^{Cdc4} acts antagonistically to the

PGC-1alpha transcriptional coactivator by targeting it for ubiquitin-mediated proteolysis. Genes Dev 22:252–264

Pietilä M, Alhonen L, Halmekyto M, Kanter P, Jänne J, Porter CW (1997) Activation of polyamine catabolism profoundly alters tissue polyamine pools and affects hair growth and female fertility in transgenic mice overexpressing spermidine/spermine N^1-acetyltransferase. J Biol Chem 272:18746–18751

Pirinen E, Kuulasmaa T, Pietilä M, Heikkinen S, Tusa M, Itkonen P, Boman S, Skommer J, Virkamäki A, Hohtola E, Kettunen M, Fatrai S, Kansanen E, Koota S, Niiranen K, Parkkinen J, Levonen AL, Ylä-Herttuala S, Hiltunen JK, Alhonen L, Smith U, Jänne J, Laakso M (2007) Enhanced polyamine catabolism alters homeostatic control of white adipose tissue mass, energy expenditure, and glucose metabolism. Mol Cell Biol 27:4953–4967

Ponugoti B, Fang S, Kemper JK (2007) Functional interaction of hepatic nuclear factor-4 and peroxisome proliferator-activated receptor-gamma coactivator 1alpha in CYP7A1 regulation is inhibited by a key lipogenic activator, sterol regulatory element-binding protein-1c. Mol Endocrinol 21:2698–2712

Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK, Lowell BB, Spiegelman BM (2001) Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. Mol Cell 8:971–982

Ratliff EP, Gutierrez A, Davis RA (2006) Transgenic expression of CYP7A1 in LDL receptor-deficient mice blocks diet-induced hypercholesterolemia. J Lipid Res 47:1513–1520

Reddy BS, Watanabe K, Weisburger JH, Wynder EL (1977) Promoting effect of bile acids in colon carcinogenesis in germ-free and conventional F344 rats. Cancer Res 37:3238–3242

Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 434:113–118

Rodgers JT, Lerin C, Gerhart-Hines Z, Puigserver P (2008) Metabolic adaptations through the PGC-1 alpha and SIRT1 pathways. FEBS Lett 582:46–53

Russell DW (1999) Nuclear orphan receptors control cholesterol catabolism. Cell 97:539–542

Russell DW, Setchell KD (1992) Bile acid biosynthesis. Biochemistry 31:4737–4749

Shin DJ, Campos JA, Gil G, Osborne TF (2003) PGC-1alpha activates CYP7A1 and bile acid biosynthesis. J Biol Chem 278:50047–50052

Spady DK, Cuthbert JA, Willard MN, Meidell RS (1995) Adenovirus-mediated transfer of a gene encoding cholesterol 7 alpha-hydroxylase into hamsters increases hepatic enzyme activity and reduces plasma total and low density lipoprotein cholesterol. J Clin Invest 96:700–709

Stieger B, Meier Y, Meier PJ (2007) The bile salt export pump. Pflugers Arch 453:611–620

Teyssier C, Ma H, Emter R, Kralli A, Stallcup MR (2005) Activation of nuclear receptor coactivator PGC-1alpha by arginine methylation. Genes Dev 19:1466–1473

Tucker JM, Murphy JT, Kisiel N, Diegelman P, Barbour KW, Davis C, Medda M, Alhonen L, Jänne J, Kramer DL, Porter CW, Berger FG (2005) Potent modulation of intestinal tumorigenesis in $Apc^{min/+}$ mice by the polyamine catabolic enzyme spermidine/spermine N^1-acetyltransferase. Cancer Res 65:5390–5398

van der Veen JN, Kruit JK, Havinga R, Baller JF, Chimini G, Lestavel S, Staels B, Groot PH, Groen AK, Kuipers F (2005) Reduced cholesterol absorption upon PPARdelta activation coincides with decreased intestinal expression of NPC1L1. J Lipid Res 46:526–534

Wang DQ, Carey MC (2003) Measurement of intestinal cholesterol absorption by plasma and fecal dual-isotope ratio, mass balance, and lymph fistula methods in the mouse: an analysis of direct versus indirect methodologies. J Lipid Res 44:1042–1059

Wang M, Tan Y, Costa RH, Holterman AX (2004) In vivo regulation of murine CYP7A1 by HNF-6: a novel mechanism for diminished CYP7A1 expression in biliary obstruction. Hepatology 40:600–608