HHS Public Access

Author manuscript

Hepatology. Author manuscript; available in PMC 2015 August 21.

Published in final edited form as:

Hepatology. 2008 December ; 48(6): 1799–1809. doi:10.1002/hep.22565.

Phosphatase and Tensin Homolog (PTEN) Regulates Hepatic Lipogenesis, Microsomal Triglyceride Transfer Protein, and the Secretion of Apolipoprotein B–Containing Lipoproteins

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Abstract

Hepatic apolipoprotein B (apoB) lipoprotein production is metabolically regulated *via* the phosphoinositide 3-kinase cascade; however, the role of the key negative regulator of this pathway, the tumor suppressor phosphatase with tensin homology (PTEN), is unknown. Here, we demonstrate that hepatic protein levels of apoB100 and microsomal triglyceride transfer protein (MTP) are significantly down-regulated (73% and 36%, respectively) in the liver of PTEN liver-specific knockout (KO) mice, and this is accompanied by increased triglyceride (TG) accumulation and lipogenic gene expression, and reduced hepatic apoB secretion in freshly isolated hepatocytes. MTP protein mass and lipid transfer activity were also significantly reduced in liver of PTEN KO mice. Overexpression of the dominant negative mutant PTEN C/S124 (adenovirus expressing PTEN C/S mutant [AdPTENC/S]) possessing constitutive phospoinositide 3-kinase activity in HepG2 cells led to significant reductions in both secreted apoB100 and cellular MTP mass (76% and 34%, respectively), and increased messenger RNA (mRNA) levels of sterol regulatory element binding protein 1c (SREBP-1c), fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC). Reduced apoB100 secretion induced by AdPTENC/S was associated with increased degradation of newly-synthesized cellular apoB100, in a lactacystin-sensitive manner, suggesting enhanced proteasomal degradation. AdPTENC/S also reduced apoB-lipoprotein production in McA-RH7777 and primary hamster hepatocytes. Our findings suggest a link between PTEN expression and hepatic production of apoB-containing lipoproteins. We postulate that perturbations in PTEN not only may influence hepatic insulin signaling and hepatic lipogenesis, but also may alter hepatic apoB-lipoprotein production and the MTP stability. On loss

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Potential conflict of interest: Nothing to report.

of PTEN activity, increased lipid substrate availability in the face of reduced hepatic lipoprotein production capacity can rapidly lead to hepatosteatosis and fatty liver.

Hepatic overproduction of apolipoprotein B (apoB)-containing very low-density lipoprotein (VLDL) particles[1] is a common complication of hepatic insulin resistance and complex perturbations in insulin signaling cascades.[1–4] In normal physiological states, insulin acutely inhibits the assembly and secretion of VLDL-apoB by posttranscriptional mechanisms.[5] Insulin was shown to inhibit apoB100 secretion through activation of phosphoinositide 3 (PI3) kinase, a pivotal molecule in the insulin action pathway.[6] Brown and Gibbons[7] suggested that insulin signaling through PI3-kinase inhibited the maturation of VLDL lipoprotein particles by preventing bulk lipid transfer to a VLDL precursor, thus enhancing the degradation of apoB. Interestingly, Akt1 (Protein Kinase B, PKBα) does not appear to be involved in acute insulin-mediated inhibition of apoB secretion,[8] suggesting that insulin signaling molecules upstream of Akt1 or in a different branch of the insulin signaling cascade may be more important in mediating control of apoB secretion. Allister et al.[9] have more recently shown that insulin-induced suppression of hepatic apoB secretion is mediated *via* activation of both the PI3 kinase and the mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) pathway.

Activation of PI3-kinase generates 3′-phospholipids including phosphatidylinositol 3,4,5-trisphosphate,[10] a lipid second-messenger molecule, which mediates many cellular responses to insulin.[11] Phosphatidylinositol 3,4,5- trisphosphate levels are attenuated by the action of a lipid phosphatase, phosphatase and tensin homolog (PTEN). Whether perturbations in PTEN can have important consequences on the overproduction of VLDL-apoB is currently unknown. Emerging evidence suggests that whole body PTEN knockout (KO) mice die at the early stages of embryonic development, which prevents study of the role of PTEN in the mammalian insulin signaling pathway in a whole animal model.[12,13] Development of PTEN liver-specific KO mice by two separate laboratories have shed important light on the potential role of PTEN in hepatic lipid and lipoprotein metabolism.[14,15] PTEN liver-specific KO mice were shown to develop fatty liver and severe steatohepatitis.[14,15] These observations suggest abnormalities in hepatic lipid and lipoprotein metabolism and led us to hypothesize that defective apoB secretion and lower microsomal triglyceride transfer protein (MTP) expression levels may be, in part, the causative factors in formation of fatty liver in PTEN liver-specific KO mice.

Materials and Methods

Animals

PTEN liver-specific KO (*Pten*$^{flox/flox}$) mice were generated as previously described.[14] Mice were fed a normal chow diet for the duration of the study, and samples were collected after a 16-hour fast.

Cell Culture and Recombinant Adenoviruses Transduction

HepG2 and HEK293 cells were purchased from ATCC (Manassas, VA). Three recombinant adenoviruses were used in this study: adenovirus expressing PTEN wild-type

(AdPTENWT), encoding full-length human wild-type PTEN complementary DNA (cDNA); AdPTENC/S, encoding a dominant negative human PTEN cDNA (cysteine 124 changed to serine within the catalytic domain),[16] and adenovirus expressing β-galactosidase (Adβ-gal), encoding β-glactosidase cDNA.

Metabolic Labeling of Adenovirus Transducted Cells and Lipoprotein Fractionation

Cells were preincubated in methionine/cysteine-free minimum essential medium in the presence or absence of 10 μM lactacystin at 37°C for 1 hour followed by pulse labeling with 50 to 100 μCi/mL [^{35}S] methionine/cysteine for 15 minutes, then chased for 0, 1, and 2 hours under the conditions described in the figure legends. For lipoprotein fractionation, conditioned media were fractionated by rate flotation ultracentrifugation.[17]

Immunoprecipitation, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis, and Fluorography

Immunoprecipitation was performed as described previously.[17] Proteins were resolved on 5% gels, fixed, and saturated with Amplify (Amersham Biosciences, Baie d'Urfë, QC) before being dried and exposed to Kodak Hyperfilm at −80°C for 1 to 4 days. Films were developed, and quantitative analysis of apoB100 bands was performed using an imaging densitometer.

Reverse Transcription Polymerase Chain Reaction Analysis of ApoB, MTP, Sterol Regulatory Element Binding Protein 1c, Acetyl-CoA Carboxylase, Fatty Acid Synthase, and Low-Density Lipoprotein-R Messenger RNAs

Total RNA was extracted using a commercially available kit (RNeasy; Qiagen). First-strand cDNA was synthesized from 5 μg total RNA using SuperScript II reverse transcriptase (Invitrogen). The resulting cDNA was subjected to 28 cycles of polymerase chain reaction amplification (denaturation at 95°C for 30 seconds; annealing at 55°C for 60 seconds; extension at 72°C for 90 seconds). The primer pairs used for detecting messenger RNA (mRNA) levels are listed in Table 1.

MTP Lipid Transfer Activity

MTP activity was performed as previously described.[18] The assay was done in Microfluor 2 Black U Bottom Microtiter plates (Thermo Labsystems, Franklin, MA). The MTP specific activity is expressed as percentage transfer per micrograms per hour.[19]

Statistical Analysis

Results are displayed as mean ± standard deviation, $n \geq 3$. The unpaired, two-tailed *t* test was used to assess differences between experimental group and controls. Probability values of less than 0.05 were considered statistically significant.

Results

PTEN Liver-specific KO Mice Have Fatty Liver, Increased Triglyceride Content, and Reduced ApoB Protein Mass

We first examined hepatic lipid and apo-lipoprotein phenotype of PTEN liver-specific KO mice and compared these with their control littermates. Similar to previous observations,[14,15] there were significant increases in total triglyceride (TG) mass in the liver from PTEN liver-specific KO mice compared with that of control mice (29.28 ±9.7 mg/g tissue, versus 5.6 ±3.6 mg/g tissue, respectively; $P < 0.01$) (data not shown). The protein levels of PTEN, apoB100, and apoB48 in the immunoblots from the liver of 13-week-old male mice with PTEN liver-specific KO were significantly decreased to 16% ±5% ($P <0.01$), 36% ±4 % ($P <0.01$), and 60% ± 5% ($P < 0.01$) of that in the control mice (Fig. 1A). The faint PTEN bands visible in the KO liver are probably attributable to nonparenchymal liver cells rather than hepatocytes.[14,15] In addition, the protein levels of apoB100 and apoB48 in the plasma from the same sets of PTEN liver-specific KO mice were also significantly decreased to 39% ± 6% ($P < 0.01$) and 56% ± 7% ($P < 0.01$) compared with that of controls (Fig. 1B). However, apolipoprotein E levels were unchanged (Fig. 1A, B). Fast protein liquid chromatography fractionation of plasma lipoproteins, followed by immunoblotting for apoB, also showed significantly lower levels of both apoB100 and apoB48 associated with plasma lipoprotein fractions of PTEN liver-specific KO mice compared with that in control littermates (Fig. 1D, E). However, plasma lipoprotein profiles were similar between the two groups (Fig. 1F). Finally, apoB secretion was measured in primary mouse hepatocytes freshly isolated from livers of PTEN liver-specific KO mice and wild-type (WT) litermates. The secretion of apoB100 and apoB48 in hepatocytes from PTEN KO was reduced by 29% ± 12% ($P < 0.05$) and 32% ±11% ($P <0.05$), respectively, compared with that in control WT littermates (Fig. 1C). Because triglyceride secretion rates were not measured in these *in vitro* experiments, we cannot rule out the possibility that more triglycerides per particle secreted from the KO hepatocytes could potentially negate the effect of decreased apoB.

Modulation of PTEN Alters Hepatic Protein Mass of ApoB100

To investigate the molecular link between PTEN and hepatic apoB, we employed HepG2 cells overexpressing either WT PTEN or dominant negative mutant PTENC/S. PTEN protein levels were significantly increased in a dose-dependent manner with AdPTENWT and AdPTENC/S adenovirus overexpression at both 5 and 20 moi (Fig. 2A, B). There was, however, a significant reduction in both secreted (45% ±3%, moi =5; 24% ±4%, moi = 20, $P <0.01$) and cellular apoB100 (47% ±13%, moi =5; 27% ± 9%, moi = 20, $P < 0.01$) in cells that were transducted with AdPTENC/S. No changes in protein level were observed in the control proteins apolipoprotein AI, apolipoprotein E, endoplasmic reticulum 60 protein (ER60), and β-actin (Fig. 2A). There were, in addition, no changes in the mass of apolipoprotein B100 (apoB100) in the cells overexpressing AdPTENWT.

PTEN Regulates Basal Activities of the Phosphoinositide 3-Kinase–AKT8 Virus Oncogene Cellular Homolog and ERK Cascades, Independent of Insulin Stimulation

There were no significant changes in the phosphorylation levels of insulin receptor beta subunit and insulin receptor substrate-1 (IRS-1) after transduction of cells with either AdPTENC/S or AdPTENWT (Fig. 3A, B). However, the phosphorylation levels of the downstream signaling effectors pyruvate dehydrogenase kinase, isozyme 1 (PDK1; Ser^{241}), AKT8 virus oncogene cellular homolog (AKT; Ser^{473} and Thr^{308}), and ERK1/2 were significantly increased by 1.32-fold ($P < 0.05$), 1.69-fold ($P < 0.05$), 2.0-fold ($P < 0.05$), and 1.26-fold ($P < 0.05$), respectively, in cells transducted with AdPTENC/S compared with Adβ-gal controls (Fig. 3C, D) [AKT(Ser473) and ERK data are not shown, as they were previously published[17]]. The increase in phosphorylation was not associated with significant changes in the protein mass (Fig. 3C, D). By contrast, when transducted with AdPTENWT, the phosphorylation levels of PDK1 (ser^{241}), AKT (Ser^{473} and Thr^{308}), and ERK1/2 were significantly reduced to 0.38-fold ($P < 0.05$), 0.21-fold ($P < 0.05$), 0.28-fold ($P < 0.05$), and 0.61-fold ($P < 0.05$) of the control, respectively, suggesting that overexpression of PTEN modulates the basal activities of both PI3K-AKT and MAPK-ERK cascades. When corrected over actin levels, there were no significant changes in the protein mass of insulin effectors measured. Interestingly, there was no insulin-stimulated ERK phosphorylation in cells overexpressing PTEN WT. Conversely, PTEN C/S mutant expression led to higher basal phosphorylation of ERK with or without insulin stimulation. Together these data appear to support the view that PTEN may negatively regulate ERK phosphorylation and that PTEN inhibition can up-regulate ERK phosphorylation. Consistently, under conditions of PTEN overexpression, HepG2 cells were found to be unresponsive to insulin treatment, which did not inhibit PTEN-induced signaling changes, nor did it cause further increases in C/S mutant expressing cells (Fig. 3C, D). It is possible that under the conditions of PTEN overexpression, downstream signaling molecules may be saturated such that HepG2 cells are unable to further respond to additional insulin stimulation. Neither secreted nor cellular apoB100 mass in HepG2 cells transducted with either PTEN recombinant adenoviruses was changed on insulin stimulation (Fig. 3E, F), suggesting that PTEN overexpression negates the insulin effect (as it acts downstream of the insulin receptor). PTEN overexpression is likely able to negate the insulin effect as it acts downstream of the insulin receptor bypassing insulin regulation.

Modulation of PTEN Alters the Secretion of Newly Synthesized ApoB100 by Affecting Intracellular ApoB100 Stability

Pulse-chase experiments were performed to assess the stability of apoB100 in HepG2 cells overexpressing WT PTEN or PTENC/S mutant. After a 15-minute pulse with 100 μCi/mL [^{35}S] methionine/ cysteine, apoB100 accumulation was monitored for up to 2 hours in the presence or absence of 10 μM lactacystin, a proteasomal inhibitor (Fig. 4A–C). Total accumulation of newly synthesized apoB100 (cellular plus secreted) was markedly increased at 1 hour (60% ± 4% versus 48% ± 2%, $P < 0.05$) and at 2 hours (60% ± 6% versus 44% ± 2%, $P < 0.05$) chase in cells transducted with AdPTENWT compared with that of Adβ-gal controls (Fig. 4D). By contrast, apoB100 accumulation was significantly decreased both at 1 hour (27% ± 5% versus 48% ± 2%, $P < 0.05$) and 2 hours (24% ± 6% versus 44% ± 2%, $P <$

0.05) chase period in cells transducted with AdPTENC/S compared with that of Adβ-gal controls. Importantly, increased apoB degradation in HepG2 cells transducted by AdPTENC/S was almost completely blocked by a proteasomal inhibitor, lactacystin treatment (Fig. 4B, D). Interestingly, this effect of lactacystin was not seen in cells overexpressing AdPTENWT, likely because of minimal degradation of apoB in these cells. Overall, these data suggest that overexpression of dominant negative PTEN C/S mutant can decrease intracellular stability of apoB100 by stimulating its proteasomal degradation in HepG2 cells, thus resulting in decreased apoB100 secretion.

Next, we examined whether modulation of PTEN affects the lipoprotein profile of apoB100-containing lipoproteins. HepG2 cells were transducted with recombinant adenoviruses and then pulse-labeled with [^{35}S]-methionine/cysteine for 3 hours in the presence of 360 μM oleate. The conditioned media were collected and subjected to density gradient ultracentrifugation to resolve the different lipoprotein subclasses. Immunoprecipitated [^{35}S]-labeled apoB100 associated with VLDL, intermedialte-density lipoprotein, and low-density lipoprotein (LDL)-sized particles was notably increased in HepG2 cells transducted with AdPTENWT (380% ± 40%, $P < 0.05$; 286% ± 50%, $P < 0.05$; and 175% ± 50%, $P < 0.05$), compared with Adβ-gal transducted controls (Fig. 4E). These data suggest that overexpression of PTEN not only may increase secretion of newly synthesized apoB100, but also may increase apoB-lipoprotein particle size (in other words, enhance lipidation of apoB particles). By contrast, HepG2 cells overexpressing AdPTENC/S secreted less VLDL-apoB100 (58% ±26% of control), intermediate-density lipoprotein-apoB100 (57% ± 24% of control), and LDL-apoB100 (63% ± 13% of control). Interestingly, apoB100 associated with small high-density lipoprotein–size particles appeared to be less affected by overexpression of PTENWT or PTENC/S mutant. Similar observations were made when experiments were performed in primary hamster hepatocytes (Fig. 4F) and McA-RH7777 (rat hepatoma cell line) (Fig. 4G), which both secrete apoB-containing VLDL particles. These data largely support the observations made in the HepG2 model and confirm that modulation of PTEN affects apoB-containing lipoproteins regardless of the cell type studied.

Expression of Sterol Regulatory Element Binding Protein 1c, Acetyl-CoA Carboxylase, Fatty Acid Synthase, ApoB and LDL-R mRNA Levels in HepG2 Cells Overexpressing PTEN or in the Liver of PTEN Liver-Specific KO Mice

The mRNA levels for a number of lipogenic genes were assessed in both HepG2 cells as well as liver of the KO mice. The mRNA level for sterol regulatory element binding protein 1c (SREBP1c), a transcriptional factor that regulates lipid synthesis, was increased both in HepG2 cells transducted with AdPTENC/S (1.56 ± 0.1-fold, $P < 0.05$, Fig. 5) and in the liver of PTEN liver-specific KO mice (1.40 ± 0.09-fold, $P < 0.01$, data not shown), similar to previous observations.[14,15] Fatty acid–modifying enzymes acting downstream of SREBP1c were also assessed. Fatty acid synthase (FAS) mRNA was significantly increased in HepG2 cells transducted with AdPTENC/S (1.44 ± 0.05-fold, $P < 0.01$, Fig. 5) and in the liver of PTEN liver-specific KO mice (1.36 ± 0.09-fold, $P < 0.01$, respectively, data not shown), similar to previous observations.[14,15] Acetyl-CoA carboxylase mRNA was slightly increased in HepG2 cells transducted with AdPTENC/S (1.13 ± 0.05-fold, not statistically significant; $P = 0.09$, Fig. 5) but was found to be significantly increased in the liver of PTEN

liver-specific KO mice (1.38 ± 0.01-fold, $P < 0.01$, data not shown), similar to previous observations.[14,15] ApoB and LDL receptor (LDL-R) mRNA levels were unchanged in both HepG2 cells transducted with AdPTENC/S and in the liver of PTEN liver-specific KO mice. The mRNA levels for SREBP1c and FAS were also assessed in HepG2 cells transducted with AdPTENC/S or AdPTENWT followed by insulin stimulation (100 nM). Insulin increased the mRNA levels for SREBP1c and FAS under basal conditions; however, there was no insulin effect on PTEN overexpression, suggesting that changes observed in SREBP1c and FAS mRNA with AdPTENC/S were insulin-stimulation independent (data not shown).

MTP Protein Mass, Lipid Transfer Activity, and mRNA Level

We also assessed the effect of PTEN on MTP, a key determinant of the assembly and secretion of apoB-containing lipoproteins. MTP protein was reduced by approximately 36% (63.8% ± 11% of the control; $P < 0.01$) in the liver of liver-specific PTEN KO mice compared with that of control mice and was decreased by approximately 37% (63.1% ± 6% of the control; n = 3, $P < 0.01$) in HepG2 cells transducted with dominant-negative AdPTENC/S (Fig. 6A). MTP enzyme activity was also decreased by approximately 25% in HepG2 cells (Fig. 6B) transducted with AdPTENC/S (75.1% ± 3% of the control; $P < 0.01$), and by approximately 15% (85% ± 6% of the control; $P < 0.05$) in the liver of liver-specific PTEN KO mice (Fig. 6B). The data suggest that modulation of PTEN can influence the MTP protein mass and lipid transfer activity (Although the effects were relatively small, chronic reductions in MTP can potentially reduce the assembly and secretion of apoB-lipoproteins.).

To determine whether PTEN modulation of MTP is exerted at the transcriptional level, we measured MTP mRNA levels. MTP mRNA level was only modestly reduced by 8% in both HepG2 cells transducted with AdPTENC/S (Fig. 6C) as well as in the liver of PTEN liver-specific KO mice (Fig. 6C), suggesting that the significant reductions in MTP protein mass may arise post-transcriptionally (for example, reduced protein stability).

Discussion

The availability of tissue-specific PTEN KO mice has led to a better understanding of the diverse biological functions of this key phosphatase.[14,21] Recent development of a liver-specific PTEN KO mouse exhibiting a phenotype of fatty liver and steatohepatitis due to increased lipogenesis and TG storage suggests a key role of this phosphatase in hepatic lipid and lipoprotein metabolism. [14,15] In the current study, we provide experimental evidence showing that reduced secretion of apoB-containing lipoproteins and enhanced lipogenesis may be the causative factors in the development of fatty liver and steatohepatitis in liver-specific PTEN KO mice. Loss of PTEN activity both in KO mice model as well as in the HepG2 cell line led to activation of lipogenesis, as evidenced from increased mRNA levels for SREBP1c, FAS, and acetyl-CoA carboxylase. This may partially explain the marked elevation in hepatic TG mass in the KO mice. The increase in hepatic lipogenesis and TG mass did not, however, lead to higher export of TG in the form of apoB-containing lipoproteins. Instead, loss of PTEN was associated with a significant reduction in apoB

secretion in the KO mice as well as three different cell culture models studied (HepG2, McA-RH7777, and primary hamster hepatocytes).

The assembly and secretion of VLDL in response to *de novo* synthesized TG is a complex process requiring coordination between the amphipathic apoB polypeptide and several classes of lipids and proteins.[20] One of the critical regulators in VLDL assembly is MTP.[18] Our findings suggest that loss of PTEN in the liver creates a state in which the PI3-kinase pathway is constitutively active, leading to chronic suppression of apoB100 and MTP protein, resulting in reduced VLDL assembly and hepatic secretion of TG. Overexpression of PTEN decreases apoB100 degradation, whereas its inhibition (through overexpression of the dominant negative PTEN C/S mutant) leads to increased degradation and decreased stability of the apoB100 protein. Because PTEN (C/S) mutant is impaired in both lipid and protein phosphatase activity, our experiments do not clearly differentiate between the metabolic effects of changes in lipid phosphatase versus protein phosphatase activities of PTEN.

PTEN activity may control the stability and cellular availability of newly synthesized hepatic apoB100 at least partially through modulation of MTP. Ample evidence suggests that inhibiting MTP activity or protein mass leads to rapid proteasomal degradation of apoB100.[22] The mechanism by which PTEN may regulate MTP levels is more difficult to delineate. Our data clearly suggest that PTEN deletion or suppression can influence MTP protein and activity levels. However, MTP mRNA was only slightly reduced. We postulate that these significant changes in MTP protein and activity levels in the PTEN KO livers, in the absence of similar changes in MTP mRNA, may be potentially explained by changes in MTP protein stability. MTP is known to be regulated by a number of nutritional and hormonal factors, including dietary fat and carbohydrate, insulin, ethanol intake, oleate treatment,[22] and the MAPK/ERK pathway.[23] Transcriptional regulation of MTP appears to be largely mediated by the MAPK/ERK pathway.[23] There was also an apparent paradox between changes in ERK1/2 phosphorylation brought about by PTEN and lack of significant changes in MTP mRNA levels, because ERK1/2 have previously been shown to regulate MTP gene transcription.[23] Our experiments show that the effect of PTEN on MTP may primarily be exerted at the protein level, although some transcriptional regulation may occur with PTEN modulation. Such a transcriptional effect is likely to be weak *in vivo,* however, because there were no significant changes in MTP mRNA levels in the livers of PTEN liver-specific KO and control mice, and in HepG2 cells infected with PTEN adenoviruses.

An important observation was that PTEN-induced alterations in apoB100 production in HepG2 cells appear to be independent of insulin stimulation. Changes in apoB100 secretion induced by PTEN activation were insensitive to insulin treatment. PTEN overexpression is likely able to negate the insulin effect as it acts downstream of the insulin receptor bypassing insulin regulation. In contrast to our findings, changes in insulin receptors and IRS-1 expression and activity have been observed after PTEN silencing by short interfering RNAs in HepG2 cells[24] and on overexpression of PTEN C/S mutant in U87MG (glioma cell line) glioblastoma cells.[25] The reasons for these conflicting observations are unclear but may relate to different experimental conditions employed. It is possible that long-term PTEN

overexpression may exert a negative feedback inhibition of upstream insulin signaling molecules and down-regulate IR and IRS expression.

A link between PTEN and hepatic insulin sensitivity as well as lipoprotein metabolism is intriguing and may be pathologically important in the development of the state of insulin resistance. PTEN overactivity can have a profound inhibitory effect on insulin signaling downstream of PI3-kinase and can readily induce insulin resistance. We have also previously reported that PTP1B (protein tyrosine phosphate 1B), a negative regulator of insulin receptor and its substrates, can influence hepatic insulin sensitivity as well as VLDL-apoB assembly and secretion.[26] We postulate that perturbations in both PTP1B and PTEN may be important components of hepatic insulin resistance and together may severely hamper signaling through the IRS-1/2-PI3 kinase-Akt cascade. Simultaneous inhibition of insulin receptor and IRS-1/-2 by PTP1B and downstream inhibition of phosphatidylinositol 3,4,5- trisphosphate generation by PTEN can severely block insulin signaling and lead to many of the metabolic consequences of insulin resistance.

In summary, PTEN may play an important role in regulation of insulin sensitivity and hepatic lipid mobilization *in vivo* and *in vitro*. Under conditions of hepatic PTEN deficiency, the PI3– kinase pathway becomes constitutively active, leading to reduced apoB availability and thus creating a block in assembly and export of hepatic lipid. Down-regulation of MTP and apoB appears to be the mechanisms by which loss of PTEN suppresses TG transport from the liver into the circulation, resulting in fatty liver (Fig. 7).

Acknowledgments

This work was supported by operating grants to K.A. (T-6041) from the Heart and Stroke Foundation of Ontario.

R.K.A. is a recipient of a RESTRA-COMP postdoctoral fellowship.

Abbreviations

AKT	AKT8 virus oncogene cellular homolog
apoB	apolipoprotein B
apoB48	apolipoprotein B48
apoB100	apolipoprotein B100
cDNA	complementary DNA
ERK	extracellular signal-regulated kinase
FAS	fatty acid synthase
IRS-1	insulin receptor substrate-1
KO	knockout
LDL	low-density lipoprotein
LDL-R	low-density lipoprotein receptor

MAPK	mitogen-activated protein kinase
mRNA	messenger RNA
MTP	microsomal triglyceride transfer protein
PDK1	pyruvate dehydrogenase kinase, isozyme 1
PI3-kinase	phosphatidylinositol-3 kinase
PTEN	phosphatase and tensin homolog
SREBP1c	sterol regulatory element binding protein 1c
TG	triglyceride
VLDL	very low density lipoprotein
WT	wild-type

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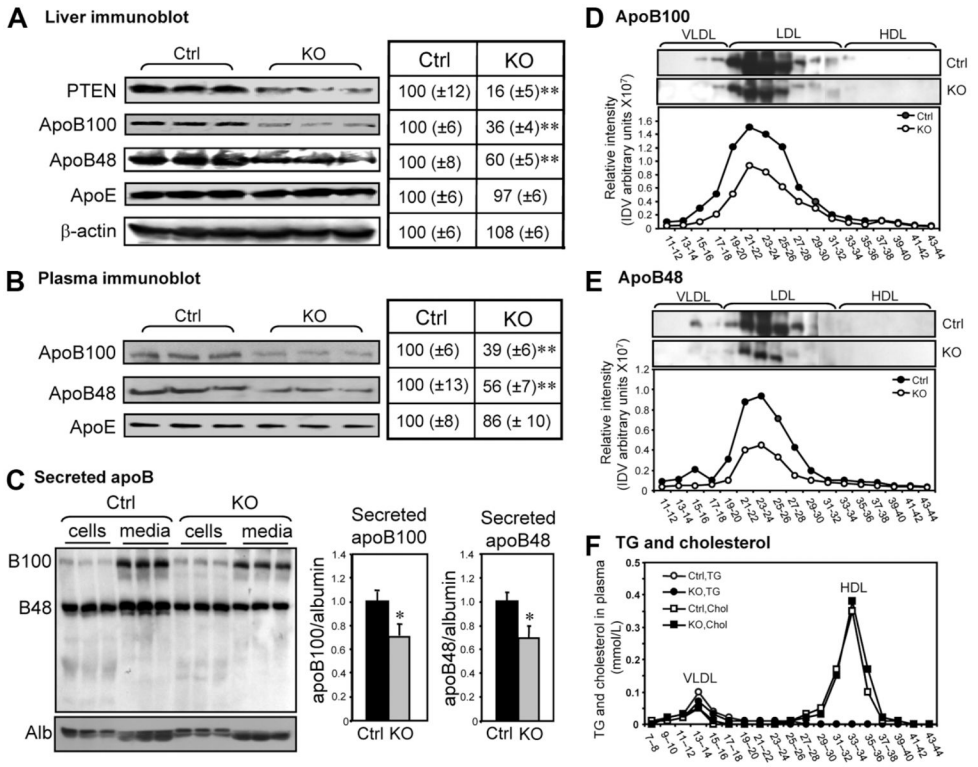


Fig. 1.

Hepatic and plasma lipid and lipoprotein profile of PTEN liver-specific KO mice. (A) Liver tissue and (B) plasma samples were analyzed by immunoblotting with antibodies against PTEN, apoB, apolipoprotein E, or *β*-actin. The data are plotted as the percentage of control mice and normalized to a control protein, *β*-actin. $^{**}P < 0.01$. (C) Secreted apoB100 and apolipoprotein B48 (apoB48) in the cultured media from primary mouse hepatocytes of control and PTEN liver-specific KO mice; n = 3, $^{*}P < 0.05$. (D) ApoB100 and (E) apoB48 levels were quantified by quantitative immunoblotting, and (F) TG and cholesterol levels in lipoprotein fractions obtained after fast protein liquid chromatography analysis of mouse plasma.

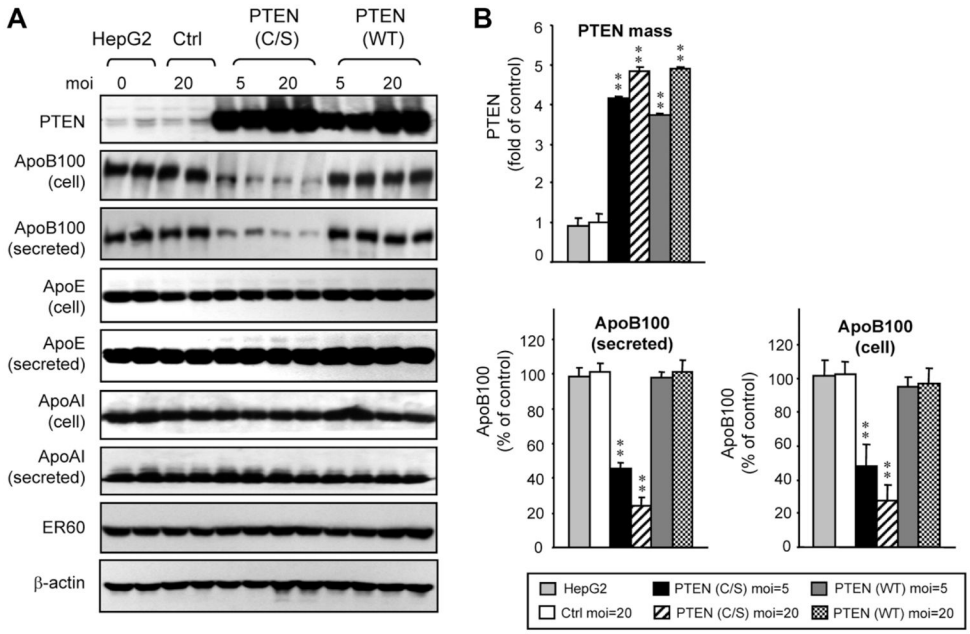


Fig. 2.

Modulation of PTEN alters hepatic protein mass of apoB100. (A) ApoB100 protein mass in control, untransducted HepG2 cells and cells transducted for 44 hours with adenoviruses encoding Ad*β*-gal (Ctrl), AdPTENC/S (C/S), and AdPTENWT (WT). Cell lysates (50 *μ*g of total cell protein) or media (16 hours media) (20 *μ*L) were then analyzed by immunoblotting. Blots show a representative experiment performed in duplicate. (B) Graphical representation of data from four independent experiments, each in duplicate or triplicate, $^{**}P < 0.01$; moi, multiplicity of infection.

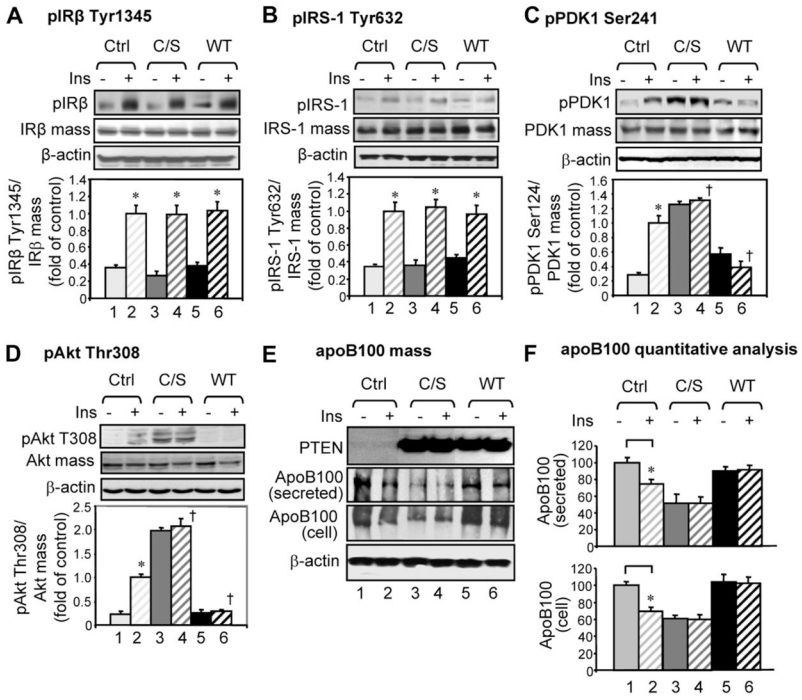
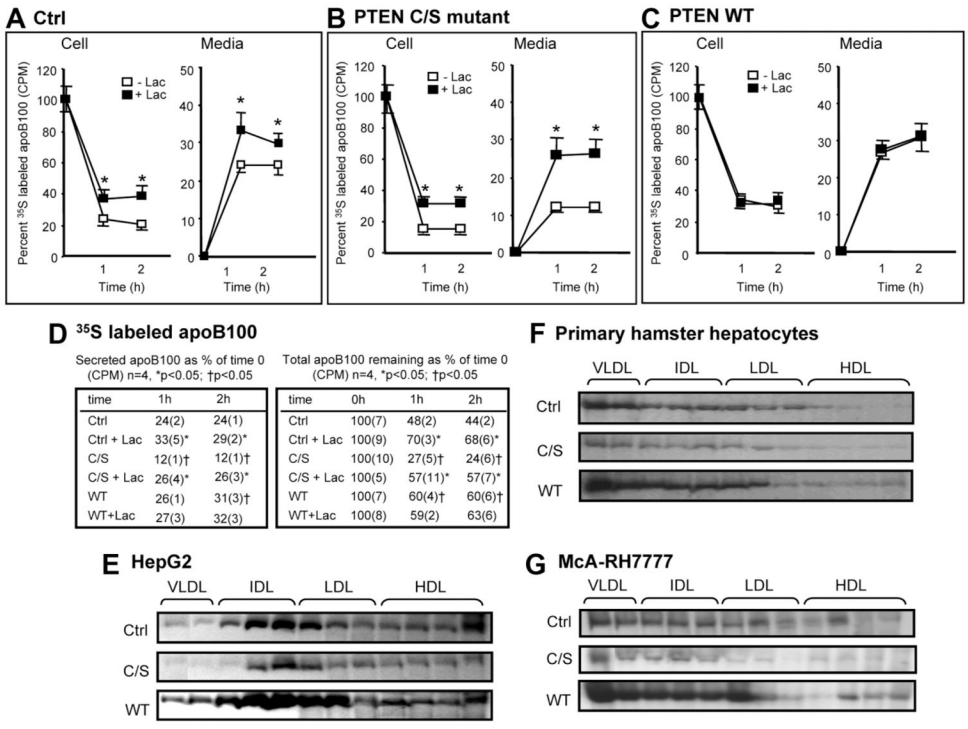


Fig. 3.

PTEN regulates basal activity of the PI3K-AKT cascade, independent of insulin stimulation or insulin receptor. HepG2 cells were pretreated with insulin-free and serum-free medium for 5 hours, followed by stimulation with 100 nM insulin for 2 to 5 minutes (A–D) or 5 hours (E). Cell lysates were analyzed by immunoblot using antibodies against phosphor-insulin receptor beta subunit (Tyr^{1345}); (A), phosphor-IRS-1 (Tyr^{632}); (B), phospho-PDK1 (Ser^{241}); (C), phospho-AKT (Thr^{308}); (D). Immunoblots were reprobed with anti-insulin receptor beta subunit (A), anti-IRS-1 (B), anti-PDK-1 (C), anti-AKT (D) antibodies. Shown in panels A–D are the ratios of phosphorylation over protein mass, (n = 3, $*P < 0.05$; $\dagger P < 0.05$). Blots were probed with antibody against PTEN or apoB (N-terminal monoclonal antibody, 1D1) (E and F); n = 3, $*P < 0.05$.



time	1h	2h
Ctrl	24(2)	24(1)
Ctrl + Lac	33(5)*	29(2)*
C/S	12(1)†	12(1)†
C/S + Lac	26(4)*	26(3)*
WT	26(1)	31(3)†
WT+Lac	27(3)	32(3)

time	0h	1h	2h
Ctrl	100(7)	48(2)	44(2)
Ctrl + Lac	100(9)	70(3)*	68(6)*
C/S	100(10)	27(5)†	24(6)†
C/S + Lac	100(5)	57(11)*	57(7)*
WT	100(7)	60(4)†	60(6)†
WT+Lac	100(8)	59(2)	63(6)

Fig. 4.

Modulation of PTEN alters the secretion of newly synthesized apoB100 by affecting intracellular apoB100 stability. Cells were then pulsed with 100 μCi/mL [^{35}S] methionine/cysteine for 15 minutes in the presence or absence of 10 μM *clasto*-Lactocystin β-*Lactone*, then chased for up to 2 hours ± lactacystin. (A–C) Immunoprecipitated [^{35}S] labeled apoB100 remaining (n = 4, *P < 0.05). [^{35}S] labeled secreted apoB100 (left panel) and total apoB100 (cellular and secreted) (right panel) were quantified under different conditions (D) (shown are means and standard error of the mean; n = 4, untreated versus lactacystin treated *P < 0.05; control Adβ-gal (Ctrl) versus AdPTENC/S or AdPTENWT †P < 0.05). Panels E–G show lipoprotein profile of apoB100-containing lipoproteins secreted by HepG2 (E), hamster hepatocytes (F), or McA-RH7777 (G) after transduction with different PTEN adenoviruses.

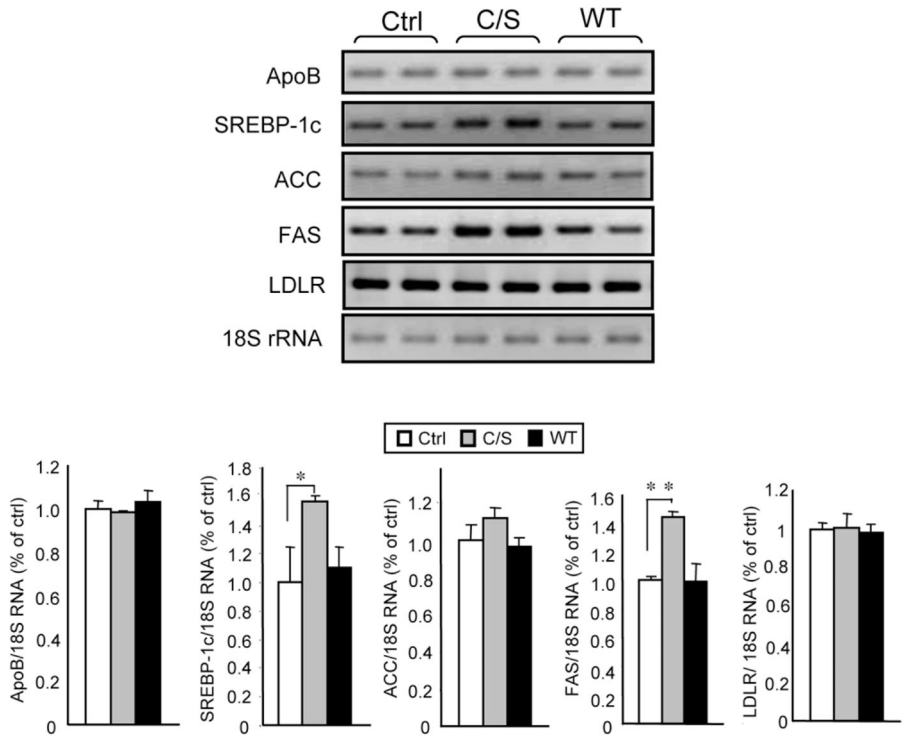


Fig. 5.

SREBP1c, acetyl-CoA carboxylase, FAS, apoB, and LDL-R mRNA levels. Messenger RNA levels were measured by reverse transcription polymerase chain reaction as described in Materials and Methods. The reverse transcription polymerase chain reaction products were quantified and expressed as the percentage of that in controls and normalized by 18S ribosomal RNA (rRNA). *$P < 0.05$, **$P < 0.01$.

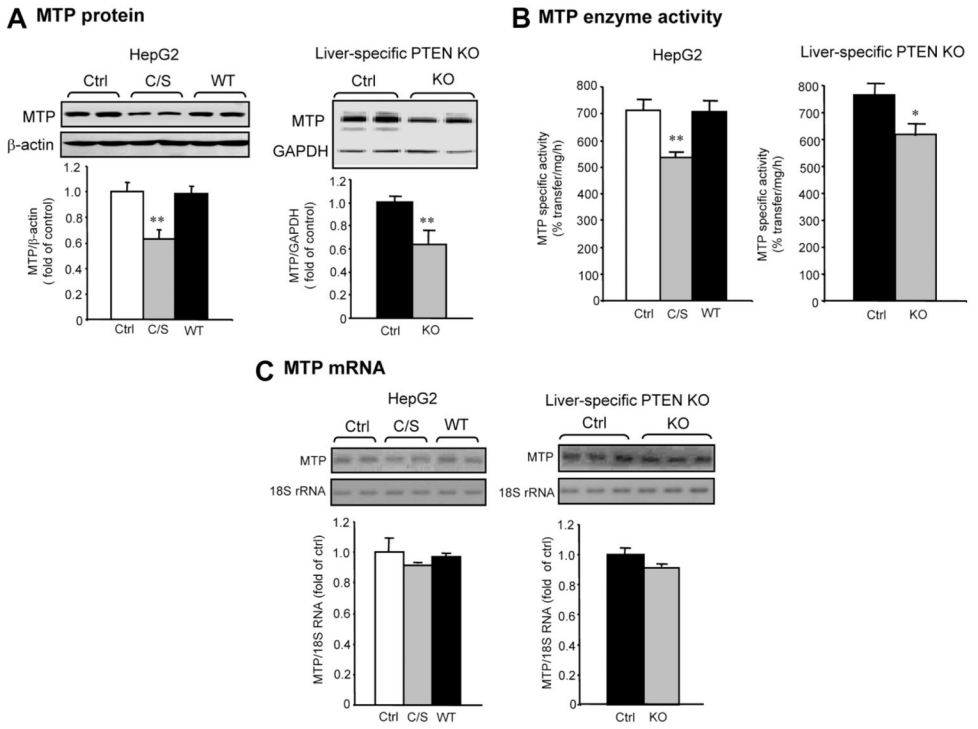


Fig. 6.

Effect of PTEN on MTP protein mass, lipid transfer activity, and mRNA. MTP protein mass was measured by western blotting from (A) HepG2 cells transducted with AdPTEN mutant (C/S) or AdPTEN (WT) (n = 3, $^{**}P < 0.01$), and liver tissue from the liver-specific PTEN KO mice (n = 6, $^{**}P < 0.01$). The MTP lipid transfer activity of HepG2 cells (B) (n = 3, $^{**}P < 0.01$) and the liver of PTEN liver-specific KO mice (B) (n = 6, $^{*}P < 0.05$), expressed as percent transfer/mg/hour. MTP mRNA levels (C) in HepG2 cells (n = 3) and in the liver-specific PTEN KO mice (n = 6).

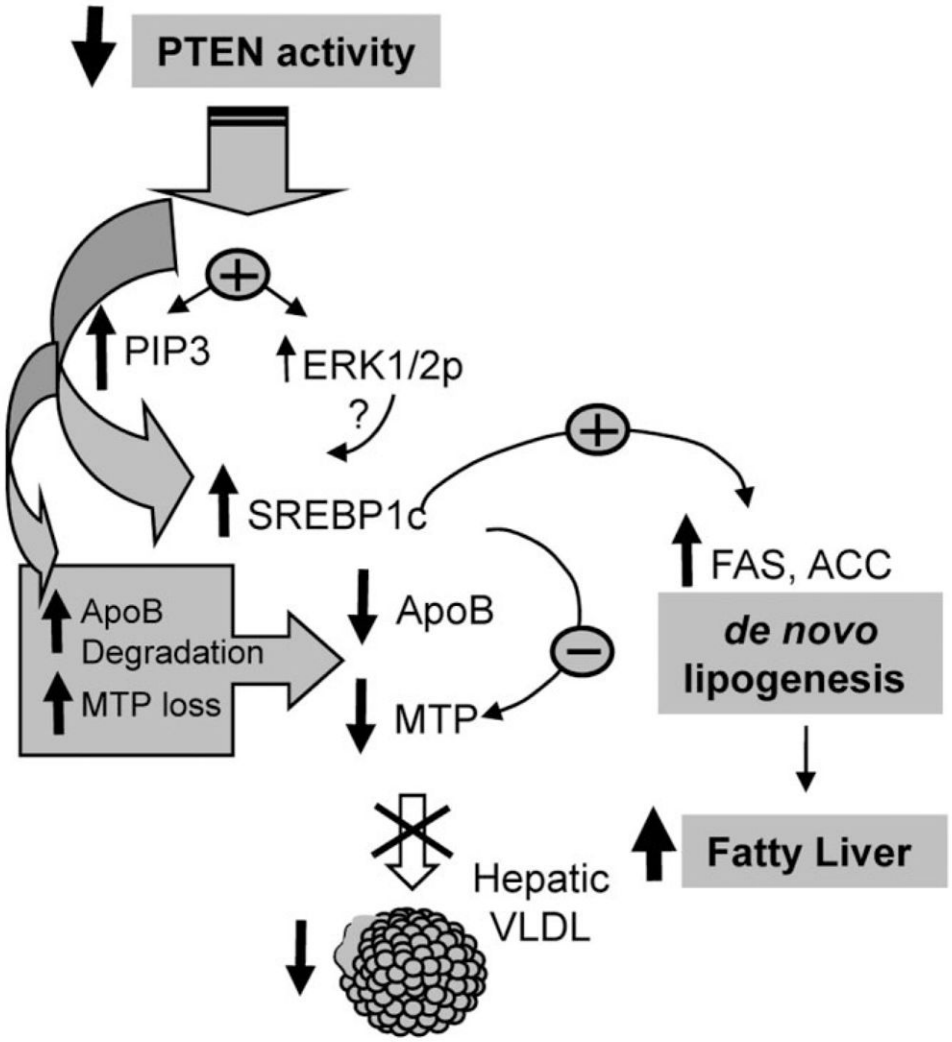


Fig. 7.

Putative mechanisms linking PTEN signaling and hepatic lipid homeostasis. Loss of PTEN activity leads to increased expression of SREBP1c and lipogenic genes, leading to enhanced *de novo* lipogenesis. Despite increased lipid substrate availability, the liver's capacity to assemble and secrete lipoproteins is also impaired because of increased apoB degradation, likely attributable to activated PI3K and ERK signaling, and reduced MTP protein mass and lipid transfer activity. Augmented lipid substrate availability in the face of reduced hepatic lipoprotein production can rapidly lead to hepatosteatosis and fatty liver.

Table 1

cDNA sequence of primers used in RT-PCR for detection of apoB, MTP, SREBP1c, ACC, FAS and LDLR mRNA levels.

	Primers for Human			
	Gene Bank # & Name		**5′ → 3′**	**Product Size (bp)**
apoB100	M14162 apoB100	F	AGACAGCATCTTCGTGTTTCAA	123
		R	ATCATTTAGTTTCAGCCCAGGA	
MTP	NM_000253 MTTP	F	TGATATTTCAGGTGCAATGGAG	54
		R	CGGGTTTTAGACTCACGATACC	
SREBP1c	NM_004176 SREBF1	F	CATCGACTACATTCGCTTTCTG	104
		R	CCAGATCCTTCAGAGATTTGCT	
ACC	NM_001093 ACACB	F	TGATCCTCTGGTTTCTTCCATT	106
		R	AGGCATTACATTCAGCATCCTT	
FAS	NM_004104 FASN	F	CTGTCTAGGTTTGATGCCTCCT	104
		R	ACGATGGCTTCATAGGTGACTT	
LDLR	AY114155 LDLR (cds)	F	GCCGTCTTTGAGGACAAAGTAT	124
		R	CCATATCCTCTGGGGACAGTAG	
18S rRNA	NR_003286	F	TAAGTCCCTGCCCTTTGTACACA	71
		R	GATCCGAGGGCCTCACTAAAC	

	Primers for Mouse			
	Gene Bank # & Name		**5′ → 3′**	**Product Size (bp)**
Apob	NM_009693 Apob	F	AAACATGCAGAGCTACTTTGGAG	121
		R	TTTAGGATCACTTCCTGGTCAAA	
Mtp	NM_008642 Mttp	F	TGGTGAAAGGGCTTATTCTGTT	53
		R	TTGCAGCTGAATATCCTGAGAA	
Srebp1c	NM_011480 Srebf1	F	ACAGTCCAGCCTTTGAGGATAG	111
		R	GACACAGAAAGGCCAGTACACA	
Acc	NM_133904 Acacb	F	CTTCCTCCTGATGAGCAACTCT	104
		R	CGTGAGTTTTCCCAAAATAAGC	
Fas	NM_007988 Fasn	F	TCAACTCACTGGCAGAAGAGAA	108
		R	CCAGAGCGTGGTTGTTAGAAAG	
Ldlr	NM_010700 Ldlr	F	TGAGGTTCCTGTCCATCTTCTT	134
		R	GGTCTTCTGGTAGACTGGGTTG	
18S rRNA	X00686	F	TAAGTCCCTGCCCTTTGTACACA	71
		R	GATCCGAGGGCCTCACTAAAC	

F, forward; R, reverse.