Angptl4 Upregulates Cholesterol Synthesis in Liver via Inhibition of LPL- and HL-Dependent Hepatic Cholesterol Uptake

Laeticia Lichtenstein, Jimmy F.P. Berbée, Susan J. van Dijk, Ko Willems van Dijk, André Bensadoun, Ido P. Kema, Peter J. Voshol, Michael Müller, Patrick C.N. Rensen and Sander Kersten

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://atvb.ahajournals.org/cgi/content/full/27/11/2420

Data Supplement (unedited) at:

http://atvb.ahajournals.org/cgi/content/full/ATVBAHA.107.151894/DC1

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at
http://atvb.ahajournals.org/subscriptions/

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:
journalpermissions@lww.com

Reprints: Information about reprints can be found online at
http://www.lww.com/reprints

Angptl4 Upregulates Cholesterol Synthesis in Liver via Inhibition of LPL- and HL-Dependent Hepatic Cholesterol Uptake

Laeticia Lichtenstein, Jimmy F.P. Berbée, Susan J. van Dijk, Ko Willems van Dijk, André Bensadoun, Ido P. Kema, Peter J. Voshol, Michael Müller, Patrick C.N. Rensen, Sander Kersten

Background—Dysregulation of plasma lipoprotein levels may increase the risk for atherosclerosis. Recently, angiopoietin-like protein 4, also known as fasting-induced adipose factor Fiaf, was uncovered as a novel modulator of plasma lipoprotein metabolism. Here we take advantage of the fasting-dependent phenotype of Angptl4-transgenic (Angptl4-Tg) mice to better characterize the metabolic function of Angptl4.

Methods and Results—In 24-hour fasted mice, Angptl4 overexpression increased plasma triglycerides (TG) by 24-fold, which was attributable to elevated VLDL-, IDL/LDL- and HDL-TG content. Angptl4 overexpression decreased post-heparin LPL activity by stimulating conversion of endothelial-bound LPL dimers to circulating LPL monomers. In fasted but not fed state, Angptl4 overexpression severely impaired LPL-dependent plasma TG and cholesteryl ester clearance and subsequent uptake of fatty acids and cholesterol into tissues. Consequently, hepatic cholesterol content was significantly decreased, leading to universal upregulation of cholesterol and fatty acid synthesis pathways and increased rate of cholesterol synthesis.

Conclusions—The hypertriglyceridemic effect of Angptl4 is attributable to inhibition of LPL-dependent VLDL lipolysis by converting LPL dimers to monomers, and Angptl4 upregulates cholesterol synthesis in liver secondary to inhibition of LPL- and HL-dependent hepatic cholesterol uptake. (*Arterioscler Thromb Vasc Biol.* 2007;27:2420-2427.)

Key Words: Angptl4 ■ lipoprotein lipase ■ cholesterol ■ triglycerides ■ liver

The enzyme lipoprotein lipase (LPL) plays a pivotal role in the regulation of plasma TG levels. By catalyzing the irreversible hydrolysis of plasma TG, LPL promotes the clearance of TG-rich lipoproteins from plasma. After synthesis in adipocytes and (cardio)myocytes, LPL protein is translocated to luminal endothelial cell surfaces where it is attached via heparan sulfate proteoglycans (HSPG).[1]

As LPL is rate-limiting for clearance of TG-rich lipoproteins, the activity of LPL is carefully regulated via numerous mechanisms. At the level of gene transcription, LPL is upregulated by the transcription factors peroxisome proliferators activated receptor (PPAR)γ in adipose tissue and by liver X receptor (LXR)α and PPARα in liver.[2] In accordance with the fat storage function of adipose tissue, expression of LPL in adipose tissue peaks in the postprandial state, whereas in skeletal muscle expression of LPL is highest in the postabsorptive state.[3] LPL is also extensively regulated at the level of enzyme activity. Indeed, several modulators of LPL activity are known, including apolipoproteins Apoc3, Apoc2,

Apoc1, and Apoa5.[4–6] In addition to apolipoproteins, recently it became evident that 2 proteins belonging to the family of angiopoietin-like proteins (Angptls), Angptl3 and Angptl4, can modulate LPL activity.[7]

In contrast to Angptl4, Angptl3 is produced exclusively in liver and is a target of LXR. It was shown that a mutation in the gene for Angptl3 is responsible for low plasma TG levels in KK/Snk mice, a mutant strain of KK obese mice.[8] The stimulatory effect of Angptl3 on fasting plasma TG can be attributed to suppression of VLDL clearance via LPL inhibition.[9]

Angptl4, also known as fasting-induced adipose factor Fiaf, was discovered by screening for genes responsive to PPARα and γ, which serve as the molecular targets for the hypolipidemic fibrate and the insulin sensitizing thiazolidinedione drugs, respectively.[10,11] Angptl4 has a molecular mass of about 50 kDa, is secreted into the blood, and may act in a paracrine and endocrine fashion. In humans, Angptl4 mRNA expression is highest in liver, followed by adipose

Figure 1. Severe hypertriglyceridemia in Angptl4-Tg mice. A, Time course of plasma TG during 24-hour fasting in WT mice (gray squares, n=7) and Angptl4-Tg mice (black squares, n=10). Error bars reflect SEM. B, Pooled plasma of 24-hour fasted WT mice (gray squares, n=7) and Angptl4-Tg mice (black squares, n=10) was analyzed for lipoprotein profiles by FPLC. Elution of molecular weight markers is indicated.

tissue, whereas in mice the order is reversed.[12] The abundance of Angptl4 in blood plasma is increased by fasting and decreased by feeding a high fat diet, suggesting a potential role in lipid metabolism.[10] Evidence linking Angptl4 to regulation of plasma lipoproteins was provided by the demonstration that injecting mice with recombinant Angptl4 protein significantly increased plasma TG concentrations, possibly by inhibiting LPL activity.[13] Subsequent studies using various models of in vivo Angptl4 overexpression or inactivation have confirmed the stimulatory effect of Angptl4 on plasma TG levels via a mechanism independent of VLDL-TG secretion.[14–18] The hypertriglyceridemic effect of Angptl4 may be modulated by formation of higher order oligomers as well as by proteolytic processing.[15] Recent in vitro studies suggest that Angptl4 inhibits LPL by promoting the conversion of catalytically active LPL dimers into catalytically inactive LPL monomers, thereby permanently inactivating LPL.[19] Currently, it is unclear whether this mechanism of LPL inhibition also occurs in vivo.

It has been suggested that in addition to lipid metabolism, Angptl4 also governs glucose homeostasis. Adenoviral-mediated Angptl4 overexpression markedly reduced plasma glucose and improved glucose tolerance.[16] However, studies with Angptl4 knockout mice failed to reveal an effect of Angptl4 on glucose homeostasis.[17] Thus, the effects of Angptl4 on glucose homeostasis remain ambiguous.

A partial analysis of the phenotype of our Angptl4 transgenic mouse model has been previously published.[18] The transgenic mice were generated using the full-length mouse genomic Angptl4 sequence containing the regulatory regions mediating responsiveness to PPARs. As a result, Angptl4 overexpression is amplified by fasting. Here we take advantage of the sensitivity of Angptl4-Tg mice to fasting to better characterize the metabolic function of Angptl4 and address: (1) the in vivo molecular mechanisms underlying its hypertriglyceridemic effect, (2) the consequence of LPL-inhibition for hepatic cholesterol metabolism and cholesterol-dependent hepatic gene regulation, and (3) the effect of Angptl4 on glucose homeostasis.

Methods

For detailed methods, please see the supplemental materials (available online at http://atvb.ahajournals.org).[20–26]

Animals

The Angptl4-transgenic mice on FVB background have been previously described.[18] Only male mice were used. All animal experiments were approved by the animal experimentation committee of Wageningen University.

Results

The Angptl4 transgenic (Angptl4-Tg) mice used in this manuscript have been previously described.[18] While in the fed state Angptl4-Tg mice show only mildly increased Angptl4 expression in skeletal muscle, heart, white adipose tissue (WAT), and brown adipose tissue (BAT), in the 24-hour fasted state dramatic Angptl4 overexpression is observed in all tissues examined, including liver (supplemental Figure I).

To examine the effect of Angptl4 overexpression on lipid metabolism, several plasma parameters were determined. Although already in the fed state Angptl4 overexpression significantly increased plasma levels of free fatty acids (FFA), glycerol, and TG, the increase was much more pronounced in the 24-hour fasted state (supplemental Table I). During the course of fasting plasma TG levels gradually decreased in WT mice, whereas plasma TG showed a marked increase in the Angptl4-Tg mice, leading to an almost 24-fold difference after 24 hours of fasting (Figure 1A). The severe hypertriglyceridemia in fasted Angptl4-Tg was mainly because of dramatically elevated plasma VLDL levels, as determined by fast protein liquid (FPLC), although IDL/LDL levels were also increased to some extent (Figure 1B). No difference in HDL-cholesterol levels was observed (Figure 1B). Detailed lipoprotein profiling was performed by gel permeation high-performance liquid chromatography (HPLC), which allows for better separation between the lipoprotein classes (supplemental Table II). In addition to VLDL, other lipoprotein fractions were also markedly enriched in TG. Amazingly, chylomicrons were still detected in plasma of 24-hour fasted Angptl4-Tg mice (supplemental Table II), which was visualized as a distinct peak in front of the VLDL peak (data not shown).

Previously, it was shown that Angptl4 inhibits the activity of LPL in vitro.[13] Using an endogenous LPL activity assay, we observed a dramatic 90% reduction in post-heparin plasma LPL activity in 24-hour fasted Angptl4-Tg compared
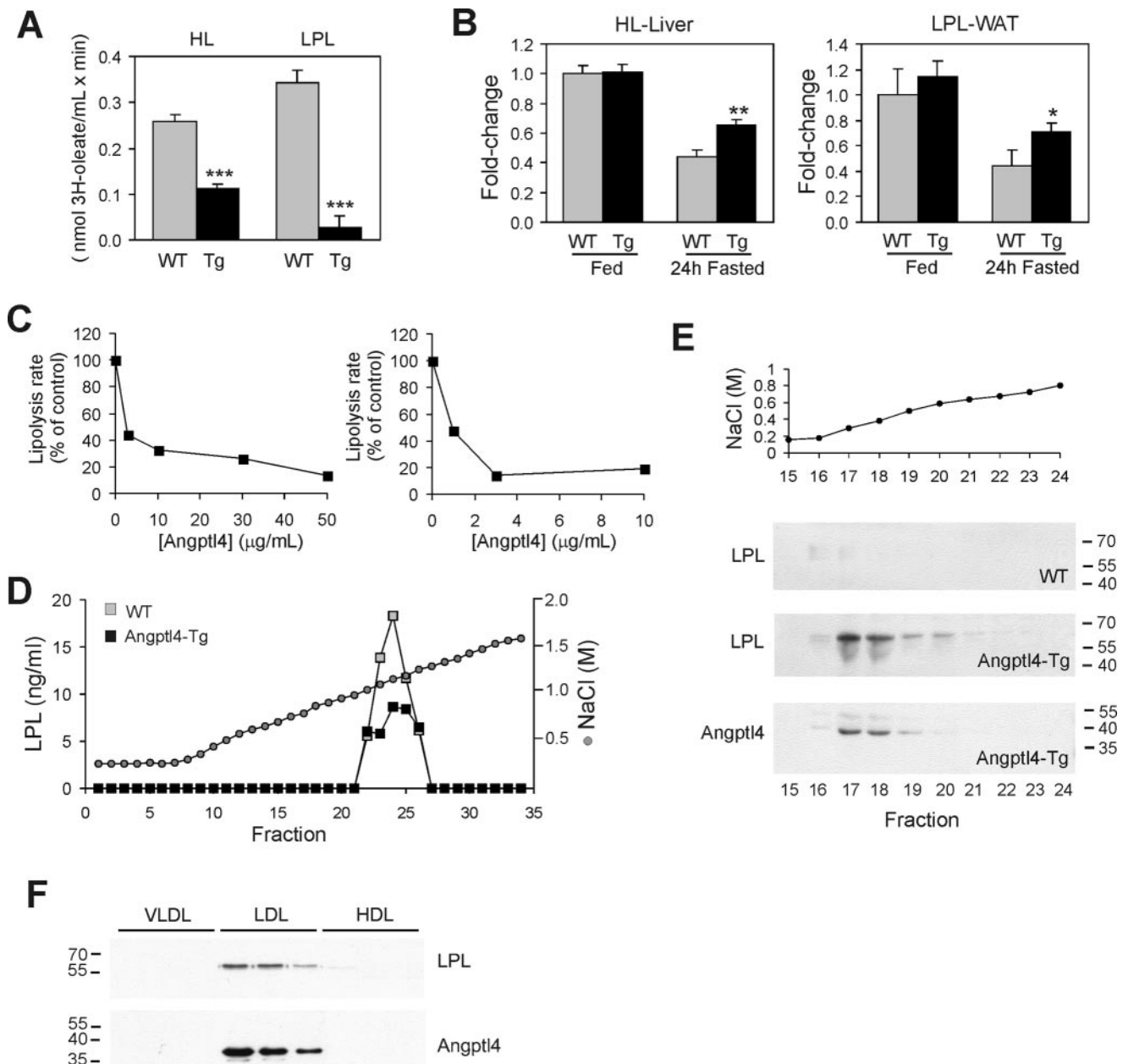
Figure 2. Angptl4 inhibits lipoprotein lipase activity by promoting formation of LPL monomers. A, Endogenous post-heparin plasma HL and LPL activity in 24-hour fasted mice (n=8 per group). B, mRNA expression of HL and LPL in liver and WAT, respectively. Errors bars represent SEM. n=7 to 10 mice per group. Statistical significance according to Student *t* test. *$P<0.05$; **$P<0.01$; ***$P<0.001$. C, Left panel: Effect of Angptl4 on LPL activity in solubilized LPL assay. Amount of [³H]FFA released at increasing concentrations of hAngptl4 is expressed as a percentage of control treatment. Right panel: Effect of Angptl4 on LPL activity in HSPG-bound LPL assay. D, Post-heparin plasma from 24-hour fasted WT and Angptl4-Tg mice was applied to a heparin sepharose column and eluted with a NaCl gradient in the presence of BSA. Fractions were analyzed for LPL by ELISA. E, Pre-heparin plasma from 24-hour fasted WT and Angptl4-Tg mice was applied to a heparin sepharose column and eluted with a NaCl gradient in the absence of BSA. Fractions were analyzed for LPL and Angptl4 by Western blot. Upper panel: NaCl concentration in fractions. F, Pooled plasma of 24-hour fasted WT mice was fractionated by FPLC. Fractions corresponding to VLDL, LDL, and HDL were analyzed for LPL or Angplt4 protein by Western blot. Molecular weight markers (in kDa) are shown.

with WT mice. Similarly, activity of hepatic lipase (HL) was about 50% reduced (Figure 2A). In contrast, mRNA levels of LPL in adipose tissue and of HL in liver were significantly increased in Angplt4-Tg mice, likely reflecting a mechanism that attempts to compensate for the decrease in enzyme activity (Figure 2B). Recent in vitro data suggest that Angptl4 inactivates LPL by promoting the conversion of catalytically active LPL dimers into inactive monomers.[19] This type of

mechanism is expected to cause permanent inactivation of LPL. To verify whether this is the case, recombinant hAngptl4 was either preincubated with [³H]TO-labeled VLDL-like emulsion particles followed by mixing with LPL and measurement of LPL activity, or alternatively preincubated with HSPG-bound LPL and removed by washing before addition of the VLDL-like particles and measurement of LPL activity. Whereas the first assay revealed an expected potent inhibition
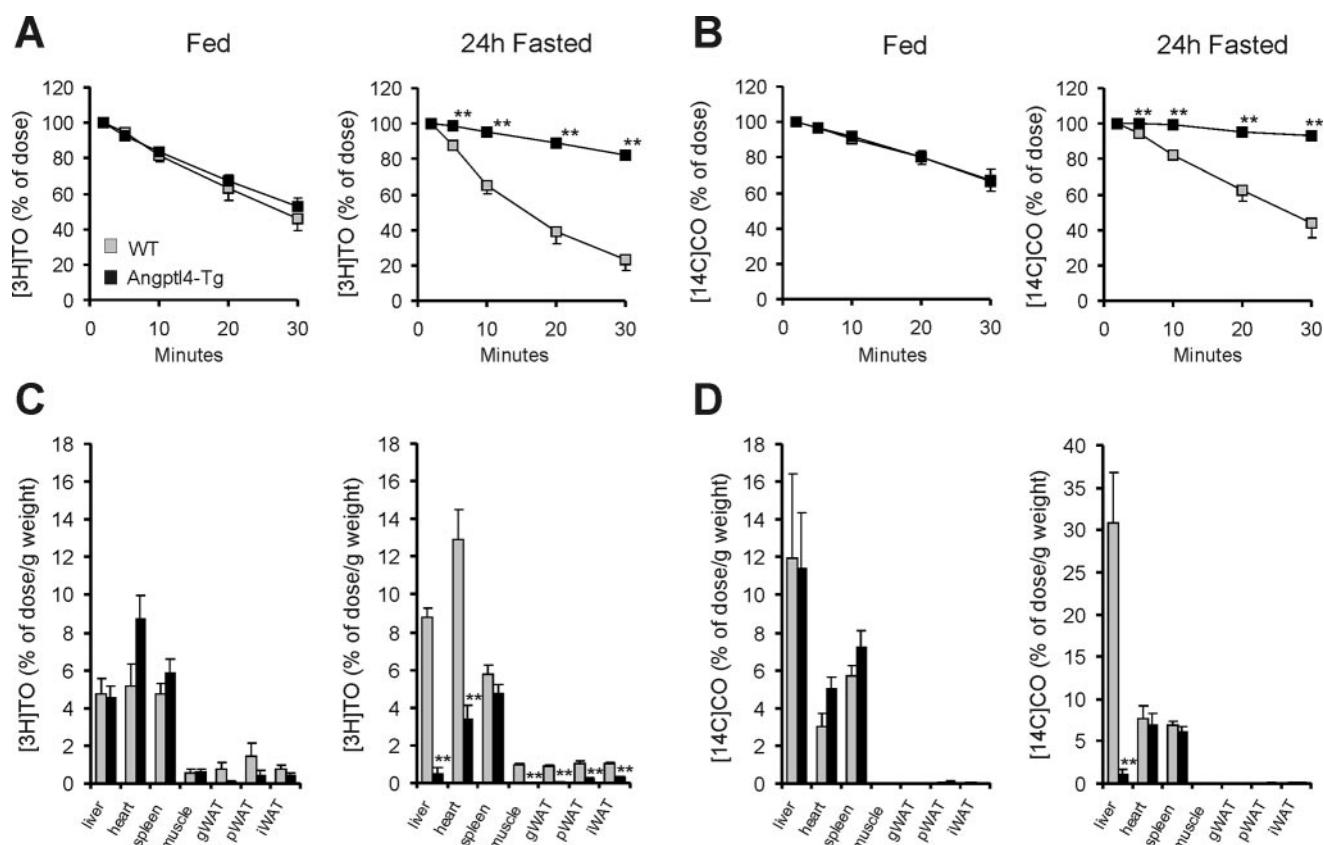
Figure 3. Serum decay and tissue uptake of radiolabeled VLDL-like emulsion particles. VLDL-like particles labeled with glycerol tri[³H]oleate and [¹⁴C]cholesteryl oleate were injected into anesthetized mice. Serum samples were collected at indicated times and measured for ³H-activity (A) and ¹⁴C-activity (B). After 30 minutes, mice were euthanized and tissues collected for measurement of ³H-activity (C) and for ¹⁴C-activity (D) (bottom). Errors bars represent SEM. n=4 to 6 mice per group. Differences between WT and Angptl4-Tg mice were evaluated by Mann–Whitney test. *$P<0.05$; **$P<0.01$.

of LPL activity by Angptl4 (Figure 2C, left panel), a similar effect was observed in the second assay (right panel), suggesting that Angptl4 may permanently disable LPL. Because LPL monomers have much lower affinity for HSPG than LPL dimers, an Angptl4-mediated LPL[27] dimer to monomer conversion would be expected to translate into a decrease in LPL dimers in Angptl4-Tg mice in post-heparin plasma, and possibly an increase in LPL monomers in pre-heparin plasma. To visualize LPL monomers and dimers, pre- and post-heparin plasma from 24-hour fasted WT and Angptl4-Tg mice was separated by heparin-sepharose affinity chromatography and fractions assayed for LPL content.[19] Importantly, a marked decrease in LPL dimer content was observed in post-heparin plasma of Angptl4-Tg mice (Figure 2D). No LPL was detected in fractions from pre-heparin plasma by ELISA (detection limit 0.2 ng/150 μL). However, using a more sensitive Western blot assay LPL protein was detected in fractions corresponding to LPL monomer in Angptl4-Tg but not WT mice (Figure 2E). These data indicate that Angptl4 overexpression promotes the conversion of endothelial-bound LPL dimers into circulating LPL monomers.

To investigate whether Angptl4 might physically associate with circulating LPL monomers, we studied the coelution of Angptl4 and LPL in pre-heparin plasma fractions from the heparin-sepharose column and gel filtration FPLC column. It was observed that Angptl4, present in the truncated form, was

present in exactly the same heparin-sepharose fractions as monomeric LPL (Figure 2E). The same observation was made in the FPLC fractions: Angptl4 was present in the fractions corresponding to (large) LDL (Figure 2F), which also harbors monomeric LPL, as previously published.[28] These data suggest that in blood plasma Angptl4 is physically associated with LDL-bound LPL monomers.

To examine whether inhibition of LPL by Angptl4 overexpression resulted in a decrease in LPL-dependent VLDL-TG lipolysis, the decay of protein-free VLDL-like emulsion particles labeled with glycerol tri[³H]oleate ([³H]TO) and [¹⁴C]cholesteryl oleate ([¹⁴C]CO) was monitored in fed and 24-hour fasted WT and Angptl4-Tg mice. In the fed state, no difference in the clearance of [³H]TO was observed between the 2 genotypes (Figure 3A; serum half life 27±4.5 minutes versus 32.2±5.2 minutes, respectively). In contrast, in the 24-hour fasted state, clearance of [³H]TO was much lower in Angptl4-Tg mice, as evidenced by an almost 8-fold increase in serum half-life (102±9.3 minutes versus 13.8±2.6 minutes, respectively; $P<0.01$). In fact, whereas in WT mice fasting accelerated [³H]TO clearance, the opposite was true in Angptl4-Tg mice. This was supported by measurement of tissue uptake of [³H]TO-derived [³H]oleate, which was markedly reduced in heart, liver, skeletal muscle, and adipose tissues of 24-hour fasted Angptl4-Tg mice in comparison with WT mice (Figure 3C). These data indicate
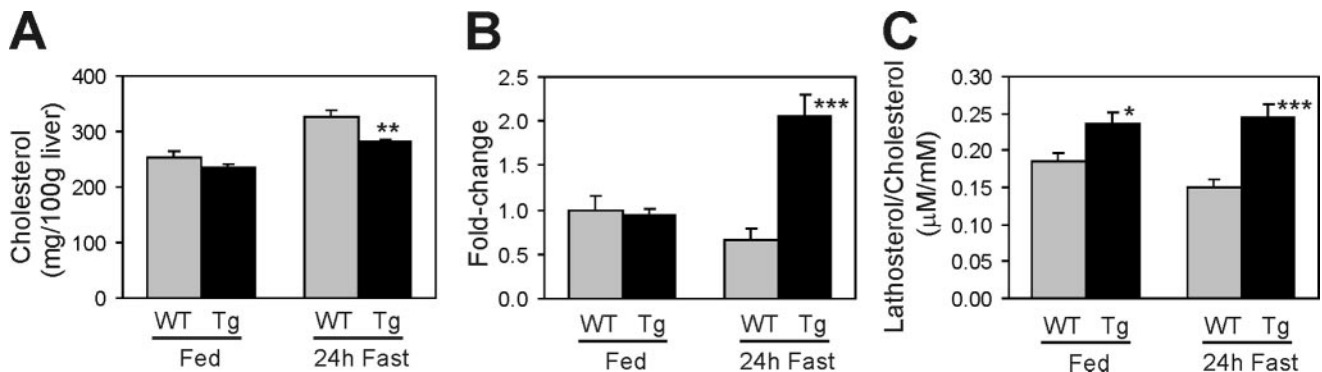
Figure 4. Upregulation of hepatic cholesterol synthesis in Angptl4-Tg mice. A, Hepatic cholesterol concentration. B, mRNA expression of 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1. C, Plasma lathosterol to cholesterol ratio. Errors bars represent SEM. n=7 to 16 mice per group. Statistical significance according to Student t test. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

that Angptl4 overexpression strongly inhibited LPL-dependent VLDL-TG lipolysis.

As the VLDL-like emulsion particles also contained [^{14}C]CO, it was possible to monitor clearance of VLDL-cholesteryl esters. Similar to the situation for [3H]TO, in WT mice fasting accelerated [^{14}C]CO clearance, whereas clearance was decelerated in Angptl4-Tg mice (Figure 3B; serum half life 29.5±10.4 minutes versus 262±24.5 minutes, respectively; $P<0.01$). Tissue uptake of [^{14}C]CO, which occurs mainly in liver and is secondary to LPL-and HL-mediated conversion of VLDL to LDL via IDL, was also strongly reduced in Angptl4-Tg mice (Figure 3D).

To find out whether the decreased hepatic uptake of VLDL [^{3}H]TO and [^{14}C]CO in Angptl4-Tg mice was paralleled by changes in liver lipids, we measured hepatic cholesterol and TG content. Whereas in fed state no difference in liver cholesterol was observed between WT and Angptl4-Tg mice, in the fasted state liver cholesterol was modestly but significantly decreased in Angptl4-Tg mice (Figure 4A). In contrast, hepatic TG content was not changed significantly in Angptl4-Tg (supplemental Figure II). In response to the decrease in hepatic cholesterol uptake and content in the fasted Angptl4-Tg mice, one would expect marked changes in the expression of genes dependent on cholesterol. To investigate the effect of Angptl4 overexpression on hepatic gene expression, we performed microarray analysis. RNA from liver of several animals was pooled and hybridized to Affymetrix mouse genome 430 2.0 GeneChip arrays. Using a cut-off of 1.4-fold, expression of a total of 103 probe sets was increased in Angptl4-Tg mice compared with WT mice. Of the 103 probe sets, 40 probe sets represented genes directly involved in cholesterol and fatty acid biosynthesis, including 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) synthase 1, HMG-CoA reductase, and Isopentenyl diphosphate δ isomerase (supplemental Figure III). Upregulation of these genes in Angptl4-Tg mice was verified by qPCR (Figure 4B and supplemental Figure IV). The increased expression of cholesterol biosynthetic enzymes at the mRNA level was supported by an increase in plasma lathosterol to cholesterol ratio, which serves as a marker for cholesterol biosynthesis rate (Figure 4C). Together, these data indicate that in the 24-hour fasted state Angptl4 overexpression causes a marked reduction in hepatic cholesterol uptake, leading to a decrease

in hepatic cholesterol content and subsequent upregulation of cholesterol biosynthesis.

Besides major changes in lipid metabolism, Angptl4 overexpression also caused alterations at the level of glucose metabolism. Whereas in the fed state no difference in plasma glucose was observed between WT and Angptl4-Tg mice, in the 24-hour fasted state Angplt4-Tg mice exhibited a modest yet significant increase in plasma glucose (Figure 5A). In contrast, no difference in plasma insulin was observed between the 2 sets of mice in either fed or fasted state (Figure 5B). These data suggest that Angptl4-Tg mice may be mildly insulin resistant. To investigate whether this is the case, we performed a hyperinsulinemic-euglycemic clamp in 24-hour fasted mice. Under basal conditions, glucose metabolism was not different between the 2 sets of mice. However, under hyperinsulinemic conditions glucose use was significantly decreased in Angptl4-Tg mice compared with WT mice (Figure 5C). Indeed, whereas in WT mice glucose use increased by 125% after insulin infusion, in Angptl4-Tg mice glucose use only went up 59% (Figure 5D). Interestingly, endogenous glucose production was not affected by insulin infusion in WT mice, characteristics of the highly insulin-resistant FVB strain, yet was clearly inhibited by insulin in Angptl4-Tg mice (Figure 5F). These data suggest that Angptl4-Tg mice are insulin-resistant in the periphery, but more insulin-sensitive at the hepatic level.

Discussion

In the present article we took advantage of the sensitivity of our Angptl4 overexpression model to fasting to better characterize the metabolic role of Angptl4. The most dramatic metabolic abnormality in Angptl4-Tg mice is the fasting-dependent elevation of plasma TG levels. The severe hypertriglyceridemia, with plasma TG levels reaching 20 mmol/L after 24 hours of fasting, can be attributed to marked inhibition of LPL-dependent VLDL and chylomicron lipolysis. Accordingly, we provide the definitive in vivo evidence that the hypertriglyceridemic effect of Angptl4 observed in several studies is attributable to impaired clearance of plasma TG via inhibition of LPL-dependent lipolysis of TG-rich lipoproteins. The inhibition of LPL activity by Angptl4 was linked to conversion of endothelial-bound catalytically active
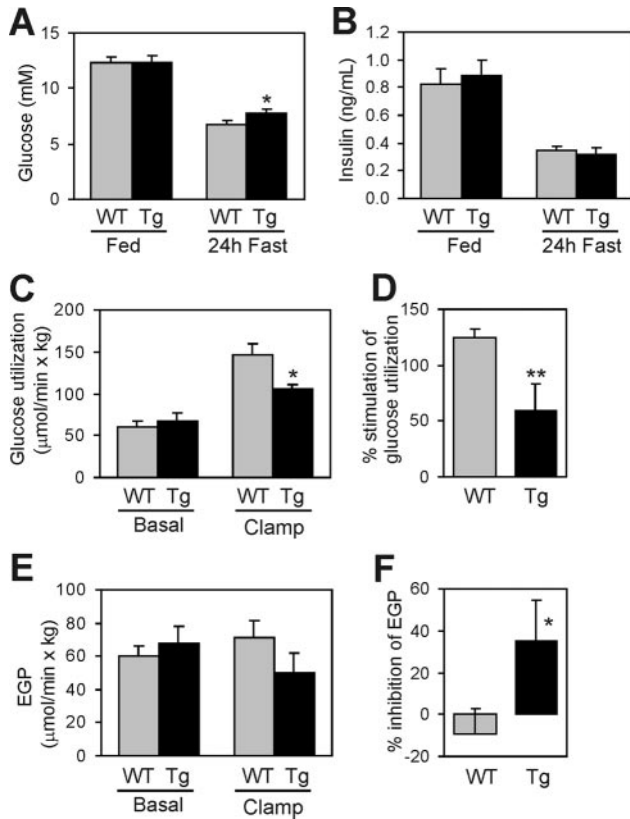
Figure 5. Peripheral insulin resistance in fasted Angptl4-Tg mice. Plasma glucose (A) and insulin (B) levels in fed and 24-hour fasted WT and Angptl4-Tg mice. n=7 to 10 mice per group. A hyperinsulinemic euglycemic clamp was carried out in 24-hour fasted WT and Angptl4-Tg mice. C, Whole body glucose use under basal and hyperinsulinemic conditions. D, Percentage induction of glucose use by insulin. E, Endogenous glucose production (EGP) under basal and hyperinsulinemic conditions. F, Percentage inhibition of EGP by insulin. Errors bars represent SEM. n=3 to 6 mice per group. Statistical significance according to Student t test. *P<0.05; **P<0.01.

LPL dimers into circulating catalytically inactive LPL monomers, as previously demonstrated in vitro.[19]

One major consequence of inhibition of LPL- and HL-dependent VLDL/IDL lipolysis in Angptl4-Tg mice is a reduced formation of LDL, leading to a marked decrease in cholesterol uptake in liver and a subsequent decrease in hepatic cholesterol content. The decrease in hepatic cholesterol led to upregulation of almost every single gene that is part of the cholesterol synthesis pathway. In fact, close to 40% of the genes identified by microarray as being upregulated in livers of Angplt4-Tg mice were involved in either fatty acid or cholesterol synthesis. Thus, by inhibiting LPL and HL, Angptl4 has a major impact on cellular cholesterol-uptake and consequently cholesterol-dependent gene regulation. The changes in gene expression translated into a functional change in the rate of cholesterol synthesis, as shown by elevated plasma lathosterol to cholesterol ratio.

We suspect that Angptl4 overexpression may similarly suppress fatty acid–dependent gene regulation, especially in tissues that rely heavily on LPL for fatty acid uptake. Fatty acid-dependent gene regulation is mainly mediated by PPARs. Previous studies have indicated that LPL can act on circulating lipoproteins to generate endogenous PPARα ligands, thus providing a potentially important link between lipoprotein metabolism and transcriptional effects of PPARα.[29] Indeed, it was shown that cardiac-specific knock-out of LPL resulted in decreased expression of several PPARα target genes.[30] Future studies will have to clarify whether Angptl4 overexpression has any influence on fatty acid and PPARα-dependent gene regulation in tissues that express LPL.

Recently, it was shown that the N-terminal domain of Angptl4 (residues 1 to 187) is able to dissociate catalytically active LPL dimers into catalytically inactive LPL monomers in vitro, suggesting a novel mechanism for regulating LPL activity.[19] Our data indicate that overexpression of Angptl4 in mice leads to conversion of endothelial-bound LPL dimers into circulating LPL monomers. Consequently, in Angptl4-Tg mice less LPL dimers are released into the circulation on heparin injection, providing an explanation for the marked decrease in post-heparin LPL activity. Interestingly, the relative decrease in post-heparin LPL activity exceeded the relative decrease in heparin-releasable LPL dimers, suggesting that Angptl4 may inhibit LPL activity via an additional mechanism. An Angptl4-mediated LPL dimer to monomer conversion was supported by the presence of LPL monomers in pre-heparin plasma of Angptl4-Tg mice but not WT mice. However, the low concentration of LPL monomers suggests that they are rapidly cleared from the circulation. Our data also suggest that in blood plasma Angptl4 is physically associated with LPL monomers, which in turn are bound to LDL. It can be hypothesized that by binding LPL monomers, Angptl4 pulls the equilibrium between LPL dimers and monomers toward monomers, which effectively results in an inhibition of LPL activity. This model conflicts with the mechanism proposed by Sukonina et al based on experiments carried out in vitro.[19] More research is necessary to elucidate the precise mechanism by which Angptl4 promotes conversion of LPL dimers to monomers in vivo.

In addition to inhibition of LPL activity, we also observed that Angptl4 overexpression resulted in a significant decrease in post-heparin plasma HL activity, although the effect was less pronounced compared with LPL. Inhibition of HL may contribute to the elevated levels of IDL/LDL, especially the increase in IDL/LDL-TG content, and likely contributes to the observed decrease in hepatic cholesterol uptake. The latter result could also be linked to increased IDL/LDL-particle size, which has been shown to lead to reduced hepatic uptake via a mechanism independent of the LDL receptor.[24]

In line with the role of HL in converting HDL2 to HDL3, inhibition of HL may contribute to the elevated HDL-TG content in Angptl4-Tg mice. Similar to LPL, HL is functionally active as a dimer.[31,32] Accordingly, it is possible that inhibition of HL activity by Angplt4 is accounted for by conversion of HL dimers to monomers. In contrast to our observations, Koster et al did not observe any change in post-heparin plasma HL activity in mice with liver-specific Angptl4 overexpression or in Angptl4 KO mice.[17] The reason for this discrepancy is not clear but may be related to differences in the sensitivity of the HL activity assay used or to differences in the magnitude of Angptl4 overexpression.

In contrast to HDL-TG, HDL-Chol levels were identical between the 2 sets of mice. Although LPL activity and HDL-Chol levels are positively correlated in humans, no link between LPL activity and HDL-Chol levels has been observed in mice overexpressing LPL or in LPL null mice, which is probably explained by the absence of CETP activity in mice.[33] Recently, it was reported that Angptl3, which is structurally and functionally highly related to Angptl4, inhibits activity of endothelial lipase, an important determinant of HDL-Chol levels.[34] Mice lacking Angptl3 showed a decrease in plasma HDL-Chol and HDL phospholipid, coupled with increased phospholipase activity in post-heparin plasma. Furthermore, Angptl3 inhibited the phospholipase activity of endothelial lipase in vitro. Whether Angptl4 may inhibit endothelial lipase as well remains to be investigated.

Whereas the impact of Angptl4 on plasma lipoprotein metabolism is well established, much less is known about the role of Angptl4 in glucose homeostasis. Recently it was reported that adenoviral-mediated overexpression of Angplt4 causes an abrupt and dramatic decrease in plasma glucose coupled to a marked improvement in glucose tolerance.[16] In contrast to the hypertriglyceridemia elicited by Angptl4 overexpression, which was transient, the hypoglycemia was persistent up to 2 weeks after virus injection when plasma levels of Angptl4 had almost returned to normal. Infection of primary hepatocytes with Angptl4 adenovirus significantly decreased secretion of glucose into the medium, suggesting that Angptl4 may lower plasma glucose by decreasing hepatic glucose output. In partial support of this notion, we find decreased endogenous glucose production in Angptl4-Tg mice under hyperinsulinemic conditions, whereas no change was found under basal conditions. At the same time, Angptl4 overexpression was associated with a decrease in insulin-mediated glucose disposal, suggesting peripheral insulin resistance. Under conditions of prolonged fasting, the effect of Angptl4 on peripheral insulin resistance seems to dominate as plasma glucose was modestly increased in Angptl4-Tg mice compared with WT mice, whereas plasma insulin levels remained unaltered. The decreased peripheral insulin sensitivity in Angptl4-Tg mice may be related to elevated plasma FFA, which are known to induce insulin resistance,[35] although a direct effect of Angptl4 protein on insulin signaling cannot be excluded. In mice, the overall impact of Angptl4 on plasma glucose levels appears to be limited, and may only be evident at higher plasma Angptl4 concentrations because inactivation of the Angptl4 did not result in any change in plasma glucose.[17] However, this observation does not exclude an effect of Angptl4 inactivation on glucose fluxes. It was reported that in humans, levels of Angptl4 in blood plasma of obese diabetics are significantly decreased in comparison with obese nondiabetics.[16] Although these data still have to be verified, they suggest that in humans Angptl4 might serve as a marker for insulin sensitivity.

Whereas the effects of Angptl4 on lipoprotein metabolism in mice are supported by numerous studies, much less is known about the role of Angptl4 in humans. Recently, it was shown that a variant of the human Angptl4 gene (E40K), which is present in approximately 3% of European Americans, was associated with significantly lower plasma TG levels and higher HDL-Chol levels.[36] These data suggest that, at least with respect to plasma TG, the effect of Angptl4 is similar between mice and humans. The picture is a little bit more complicated for HDL-Chol. Previously, we found a positive correlation between plasma Angptl4 concentration, assessed semiquantitatively by immunoblot, and HDL-Chol levels.[23] In the present study, no change in plasma HDL-Chol level was observed in Angptl4-Tg mice, despite a >20-fold increase in plasma TG. Thus, the link between Angptl4 and HDL, and the impact of Angptl4 on effectors of plasma HDL levels, deserve further study.

In conclusion, we show that (1) the hypertriglyceridemic effect of Angptl4 is attributable to inhibition of LPL-dependent VLDL lipolysis by converting endothelial-bound LPL dimers to circulating LPL monomers, (2) Angptl4 upregulates cholesterol synthesis in liver via inhibition of LPL- and HL-dependent hepatic cholesterol uptake, and (3) Angptl4 overexpression increases insulin sensitivity in liver but decreases insulin sensitivity in the periphery.

Disclosures
None.

References
1. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. *J Lipid Res*. 2002;43:1997–2006.
2. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J. PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *Embo J*. 1996;15:5336–5348.
3. Olivecrona T, Bergo M, Hultin M, Olivecrona G. Nutritional regulation of lipoprotein lipase. *Can J Cardiol*. 1995;11 Suppl G:73G–78G.
4. Berbee JF, van der Hoogt CC, Sundararaman D, Havekes LM, Rensen PC. Severe hypertriglyceridemia in human APOC1 transgenic mice is caused by apoC-I-induced inhibition of LPL. *J Lipid Res*. 2005;46:297–306.
5. van Dijk KW, Rensen PC, Voshol PJ, Havekes LM. The role and mode of action of apolipoproteins CIII and AV: synergistic actors in triglyceride metabolism? *Curr Opin Lipidol*. 2004;15:239–246.
6. Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. *Arterioscler Thromb Vasc Biol*. 1999;19:472–484.
7. Kersten S. Regulation of lipid metabolism via angiopoietin-like proteins. *Biochem Soc Trans*. 2005;33:1059–1062.
8. Koishi R, Ando Y, Ono M, Shimamura M, Yasumo H, Fujiwara T, Horikoshi H, Furukawa H. Angptl3 regulates lipid metabolism in mice. *Nat Genet*. 2002;30:151–157.
9. Shimizugawa T, Ono M, Shimamura M, Yoshida K, Ando Y, Koishi R, Ueda K, Inaba T, Minekura H, Kohama T, Furukawa H. ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. *J Biol Chem*. 2002;277:33742–33748.
10. Kersten S, Mandard S, Tan NS, Escher P, Metzger D, Chambon P, Gonzalez FJ, Desvergne B, Wahli W. Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. *J Biol Chem*. 2000;275:28488–28493.
11. Yoon JC, Chickering TW, Rosen ED, Dussault B, Qin Y, Soukas A, Friedman JM, Holmes WE, Spiegelman BM. Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. *Mol Cell Biol*. 2000;20:5343–5349.

12. Zandbergen F, van Dijk S, Muller M, Kersten S. Fasting-induced adipose factor/ angiopoietin-like protein 4: a potential target for dyslipidemia? *Future Lipidology*. 2006;1:227–236.

13. Yoshida K, Shimizugawa T, Ono M, Furukawa H. Angiopoietin-like protein 4 is a potent hyperlipidemia-inducing factor in mice and inhibitor of lipoprotein lipase. *J Lipid Res*. 2002;43:1770–1772.

14. Yu X, Burgess SC, Ge H, Wong KK, Nassem RH, Garry DJ, Sherry AD, Malloy CR, Berger JP, Li C. Inhibition of cardiac lipoprotein utilization by transgenic overexpression of Angptl4 in the heart. *Proc Natl Acad Sci U S A*. 2005;102:1767–1772.

15. Ge H, Yang G, Yu X, Pourbahrami T, Li C. Oligomerization state-dependent hyperlipidemic effect of angiopoietin-like protein 4. *J Lipid Res*. 2004;45:2071–2079.

16. Xu A, Lam MC, Chan KW, Wang Y, Zhang J, Hoo RL, Xu JY, Chen B, Chow WS, Tso AW, Lam KS. Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. *Proc Natl Acad Sci U S A*. 2005;102: 6086–6091.

17. Koster A, Chao YB, Mosior M, Ford A, Gonzalez-DeWhitt PA, Hale JE, Li D, Qiu Y, Fraser CC, Yang DD, Heuer JG, Jaskunas SR, Eacho P. Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism. *Endocrinology*. 2005;146:4943–4950.

18. Mandard S, Zandbergen F, van Straten E, Wahli W, Kuipers F, Muller M, Kersten S. The fasting-induced adipose factor/angiopoietin-like protein 4 is physically associated with lipoproteins and governs plasma lipid levels and adiposity. *J Biol Chem*. 2006;281:934–944.

19. Sukonina V, Lookene A, Olivecrona T, Olivecrona G. Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc Natl Acad Sci U S A*. 2006;103: 17450–17455.

20. Thompson RH, Merola GV. A simplified alternative to the AOAC official method for cholesterol in multicomponent foods. *J AOAC Int*. 1993;76: 1057–1068.

21. Duivenvoorden I, Teusink B, Rensen PC, Romijn JA, Havekes LM, Voshol PJ. Apolipoprotein C3 deficiency results in diet-induced obesity and aggravated insulin resistance in mice. *Diabetes*. 2005;54:664–671.

22. van Vlijmen BJ, Rohlmann A, Page ST, Bensadoun A, Bos IS, van Berkel TJ, Havekes LM, Herz J. An extrahepatic receptor-associated protein-sensitive mechanism is involved in the metabolism of triglyceride-rich lipoproteins. *J Biol Chem*. 1999;274:35219–35226.

23. Mandard S, Zandbergen F, Tan NS, Escher P, Patsouris D, Koenig W, Kleemann R, Bakker A, Veenman F, Wahli W, Muller M, Kersten S. The direct peroxisome proliferator-activated receptor target fasting-induced adipose factor (FIAF/PGAR/ANGPTL4) is present in blood plasma as a truncated protein that is increased by fenofibrate treatment. *J Biol Chem*. 2004;279:34411–34420.

24. Rensen PC, Herijgers N, Netscher MH, Meskers SC, van Eck M, van Berkel TJ. Particle size determines the specificity of apolipoprotein E-containing triglyceride-rich emulsions for the LDL receptor versus hepatic remnant receptor in vivo. *J Lipid Res*. 1997;38:1070–1084.

25. Jong MC, Rensen PC, Dahlmans VE, van der Boom H, van Berkel TJ, Havekes LM. Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. *J Lipid Res*. 2001;42:1578–1585.

26. Voshol PJ, Haemmerle G, Ouwens DM, Zimmermann R, Zechner R, Teusink B, Maassen JA, Havekes LM, Romijn JA. Increased hepatic insulin sensitivity together with decreased hepatic triglyceride stores in hormone-sensitive lipase-deficient mice. *Endocrinology*. 2003;144: 3456–3462.

27. Van Tilbeurgh H, Roussel A, Lalouel JM, Cambillan C. Lipoprotein lipase molecular model based on the pancreatic lipase x-ray structure: consequences for heparin binding and catalysis. *J Biol Chem*. 2004;269: 4626–4633.

28. Vilella E, Joven J, Fernandez M, Vilaro S, Brunzell JD, Olivecrona T, Bengtsson-Olivecrona G. Lipoprotein lipase in human plasma is mainly inactive and associated with cholesterol-rich lipoproteins. *J Lipid Res*. 1993;34:1555–1564.

29. Ziouzenkova O, Perrey S, Asatryan L, Hwang J, MacNaul KL, Moller DE, Rader DJ, Sevanian A, Zechner R, Hoefler G, Plutzky J. Lipolysis of triglyceride-rich lipoproteins generates PPAR ligands: evidence for an antiinflammatory role for lipoprotein lipase. *Proc Natl Acad Sci U S A*. 2003;100:2730–2735.

30. Augustus A, Yagyu H, Haemmerle G, Bensadoun A, Vikramadithyan RK, Park SY, Kim JK, Zechner R, Goldberg IJ. Cardiac-specific knock-out of lipoprotein lipase alters plasma lipoprotein triglyceride metabolism and cardiac gene expression. *J Biol Chem*. 2004;279: 25050–25057.

31. Hill JS, Davis RC, Yang D, Wen J, Philo JS, Poon PH, Phillips ML, Kempner ES, Wong H. Human hepatic lipase subunit structure determination. *J Biol Chem*. 1996;271:22931–22936.

32. Berryman DE, Mulero JJ, Hughes LB, Brasaemle DL, Bensadoun A. Oligomeric structure of hepatic lipase: evidence from a novel epitope tag technique. *Biochim Biophys Acta*. 1998;1382:217–229.

33. Clee SM, Zhang H, Bissada N, Miao L, Ehrenborg E, Benlian P, Shen GX, Angel A, LeBoeuf RC, Hayden MR. Relationship between lipoprotein lipase and high density lipoprotein cholesterol in mice: modulation by cholesteryl ester transfer protein and dietary status. *J Lipid Res*. 1997;38:2079–2089.

34. Shimamura M, Matsuda M, Yasumo H, Okazaki M, Fujimoto K, Kono K, Shimizugawa T, Ando Y, Koishi R, Kohama T, Sakai N, Kotani K, Komuro R, Ishida T, Hirata K, Yamashita S, Furukawa H, Shimomura I. Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase. *Arterioscler Thromb Vasc Biol*. 2007; 27:366–372.

35. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 2006;444:840–846.

36. Romeo S, Pennacchio LA, Fu Y, Boerwinkle E, Tybjaerg-Hansen A, Hobbs HH, Cohen JC. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. *Nat Genet*. 2007;39:513–516.

SUPPLEMENTAL METHODS

Animal experiments. The Angptl4-transgenic mice on FVB background have been previously described [18]. Only male mice were used. Animals were kept in standard cages with food and water ad libitum. Animals were fed normal laboratory chow (RMH-B 2181, Arie Blok animal feed, Woerden, the Netherlands), containing 23.5% protein, 5% fat, 38.3% starch, and 4% sugar. Mice in fed state were sacrificed at the beginning of the light cycle. Mice in fasted state were deprived of food for 24 hours starting at the beginning of the light cycle. At the time of sacrifice animals were between 2 and 4 months of age. Blood was collected via orbital puncture into EDTA tubes. After sacrificing the mice by cervical dislocation, tissues were excised and immediately frozen in liquid nitrogen. All animal experiments were approved by the animal experimentation committee of Wageningen University.

Plasma metabolites. Blood was collected into paraoxon-coated capillaries to prevent ongoing in vitro lipolysis. Blood samples were placed on ice and centrifuged at 4°C for 10 minutes at 10,000 g. The plasma glucose concentration was determined using a kit from Elitech (Sopachem, Wageningen, Netherlands). Plasma and tissue TG and plasma glycerol concentrations were determined using kits from Instruchemie (Delfzijl, Netherlands). Plasma free fatty acids were determined using a kit from WAKO Chemicals (Sopachem, Wageningen, Netherlands). Plasma ketones (β-hydroxybutyrate) were determined using Precision Xtra (Abbott laboratories, Bedford, MA, USA). plasma lathosterol was determined by capillary gas chromatography. Plasma insulin was determined using a kit from Linco (St. Charles, MO, USA).

Liver cholesterol. Liver cholesterol content was determined as previously described [20]. Briefly, a ~100 mg liver samples was saponified by heating in ethanol-KOH, and cholesterol was extracted from the saponified solution with hexane. After evaporation of hexane, cholesterol was derivatized to trimethylesters using bis-silyl-tri-fluor-aceetamide. Cholesterol was subsequently quantified by gas chromatography.

Lipoprotein profiling. Plasma lipoproteins of wild-type and Angptl4-Tg mice were separated using fast protein liquid chromatography (FPLC). 0.2 mL of pooled plasma was injected into a Superose 6B 10/300 column (GE Healthcare Bio-Sciences AB, Roosendaal, Netherlands) and eluted at a constant flow of 0.5 mL/minute with phosphate buffered saline (pH 7.4). The effluent was collected in 0.5 mL fractions and TG and cholesterol levels were determined (Instruchemie). Separation of lipoproteins by gel permeation HPLC was performed as a lipoprotein analysis service by LipoSEARCH (Tokyo, Japan) using 10 μL of pooled plasma.

Modulated plasma LPL and HL activity assay. 24 hour fasted mice were injected via the tail vein with heparin (0.1U/g; Leo Pharmaceutical Products B.V., Weesp, Netherlands) and blood was collected after 10 min. The plasma was snap-frozen and stored at -80°C until analysis of LPL and hepatic lipase (HL) activity as previously described [21].

In vitro LPL activity assay. The effect of recombinant hAngptl4 (residues 26-229, hAngptl-S2) (Biovendor Laboratory Medicine, Modrice, Czech Republic) on LPL-dependent TG hydrolysis of [³H]TO-VLDL-like emulsion particles was determined as described (solubilized LPL assay) [4]. A HSPG-bound LPL assay was performed by first coating plates with HSPG (250 ng/well), followed by coating with LPL (0.2 U/well). HSPG-bound LPL was subsequently pre-incubated with recombinant hAngptl4 for 30 min at 37°C, washed three times with 100 mM Tris-HCl pH 8.5, followed by addition of [³H]TO-VLDL-like emulsion

particles. After 15 minutes incubation, the reaction was terminated and lipids extracted as described[4]. The amount of [^{3}H]FFA released was measured by scintillation counting and expressed as a percentage of [^{3}H]FFA released in the absence of Angptl4.

Heparin-Sepharose Chromatography. Heparin-sepharose chromatography was carried out on a Pharmacia FPLC system. A HiTrap Heparin HP 1 mL column (GE Healthcare Bio-Sciences AB, Roosendaal, Netherlands) was equilibrated with 0.25M NaCl/20% glycerol/1% BSA/10 mM sodium phosphate pH6.5. Pre- and post-heparin plasma was adjusted to the concentration of the equilibration buffer before loading. After loading the equivalent of 0.2 ml mouse blood plasma, the column was washed (10mL), followed by elution with a linear gradient of NaCl (from 0.25 to 1.5 M) in equilibration buffer at a flow rate of 0.5 ml/minute. BSA was omitted when fractions were intended for analysis by Western blot. Murine LPL in fractions was measured by enzyme-linked immunosorbent assay [22].

Isolation of total RNA and Q-PCR. Extraction of RNA, cDNA synthesis and quantitative PCR were carried out as previously described [18].

Western blot. Fractions collected of the HiTrap HP-column or Superose 6B column were separated by SDS-PAGE and transferred to Immobilon-P membrane (Millipore, Bedford, MA, USA). Western blotting was carried out as described previously [10], [23]. The LPL primary antibody (anti-mouse LPL CA1787, Cell Applications Inc., San Diego, CA, USA) was used at 1:500, and the secondary antibody (HRP-labelled anti-rabbit IgG, Sigma) at 1:4000. All incubations were performed in Tris-buffered saline, pH 7.5, with 0.1% Tween-20 and 5% dry milk, except for the final washings when dry milk was omitted.

Preparation of VLDL-like Emulsion Particles. TG-rich VLDL-like emulsion particles (80 nm) were prepared as described [24]. Radiolabeled emulsions were obtained by adding 200 µCi of glycerol tri[^{3}H]oleate (triolein, TO) and 20 µCi of [^{14}C]cholesteryl oleate (CO) to 100 mg of emulsion lipids before sonication.

In Vivo Clearance of VLDL-Like Emulsion Particles. Mice were anesthetized with hypnorm(fentanyl)/midazolam and injected with the radiolabeled emulsion particles (1.0 mg TG in 200 µl PBS) at 8:00 a.m. into the tail vein. At indicated time points after injection, blood was taken from the tail vein to determine the serum decay of [^{3}H]TO and [^{14}C]CO by scintillation counting (Packard Instruments, Dowers Grove, IL, USA). At 30 min after injection, mice were sacrificed by cervical dislocation and tissues were collected. Tissues were weighed and dissolved overnight in Solvable (Packard Bioscience, Meriden, CT, USA), followed by measurement of ^{3}H- and ^{14}C-activities in Ultima Gold (Packard Bioscience). The total plasma volumes of the mice were calculated from the equation V (ml) = 0.04706 x body weight (g), as determined from ^{125}I-BSA clearance studies as previously described [25].

Microarray. RNA was prepared from livers of 24h fasted wild-type and Angptl4-Tg mice using Trizol and subsequently pooled per group (n=7). Pooled RNA was further purified using Qiagen RNeasy columns and the quality verified by lab on a chip analysis (Bioanalyzer 2100, Agilent, Amsterdam, Netherlands). 10 µg of RNA was used for one cycle of cRNA synthesis (Affymetrix, Santa Clara, USA). Hybridization, washing and scanning of Affymetrix Genechip mouse genome 430 2.0 arrays was according to standard Affymetrix protocols. Fluorimetric data were processed by Affymetrix GeneChip Operating software and the gene chips were globally scaled to all the probe sets with an identical target intensity value

using MAS5.0 algorithm. Further analysis was performed by Data Mining Tool (Affymetrix). Only probe sets labeled present in both wild-type and Angptl4-Tg mice were considered.

Hyperinsulinemic euglycemic clamp analysis. Wild-type and Angptl4-Tg mice were fasted for 24 hours prior to the clamp studies. The hyperinsulinemic euglycemic clamp and assays for blood glucose and plasma insulin were carried out as previously described [26].

Supplemental Table I:**Plasma parameters.** FFA, glycerol, ketones bodies and TG concentrations were determined in EDTA plasma of fed and 24h fasted WT and Angptl4-Tg mice. Values are mean±SEM. n=7-10 mice/group. Statistical significance according to Student's t test. *, p< 0.05; **, p< 0.01.

	FFA (mM)	Glycerol (mM)	Ketones (mM)	TG (mM)
Fed				
Wild-type	0.40±0.02	0.24±0.01	n.d.	1.79±0.15
Angptl4-Tg	0.57±0.05**	0.33±0.04*	n.d.	3.36±0.64**
Fast 24h				
Wild-type	0.58±0.04	0.31±0.02	1.20±0.07	0.74±0.07
Angptl4-Tg	1.32±0.09**	0.99±0.09**	1.38±0.06*	17.58±2.22**

Supplemental Table II: **Lipoprotein cholesterol and TG content.** Pooled plasma of 24h fasted wild-type and Angptl4-Tg mice was separated by gel permeation HPLC using lipoprotein analysis service by LipoSEARCH. CM, chylomicron. Concentrations are expressed in mM. n=7-10 mice/group.

	Total Cholesterol	CM Cholesterol	VLDL Cholesterol	IDL/LDL Cholesterol	HDL Cholesterol	Total Triglyceride	CM Triglyceride	VLDL Triglyceride	IDL/LDL Triglyceride	HDL Triglyceride	Free Glycerol
Wild-type	3.91	0.00	0.15	0.42	3.33	0.61	0.00	0.36	0.22	0.04	0.28
Angptl4-Tg	5.91	0.23	1.69	0.81	3.19	12.57	1.61	9.09	1.40	0.46	0.82

Supplemental Figure I: **Marked over-expression of Angptl4 mRNA in 24h fasted Angptl4-Tg mice.** Angptl4 mRNA expression in liver, heart, white adipose tissue (WAT), skeletal muscle, and brown adipose tissue (BAT) of fed and 24h fasted WT (grey bars) and Angptl4-Tg (black bars) mice. Errors bars represent SEM. n=5-10 mice/group. Statistical significance according to Student's t test. **, p< 0.01; ***, p< 0.001.
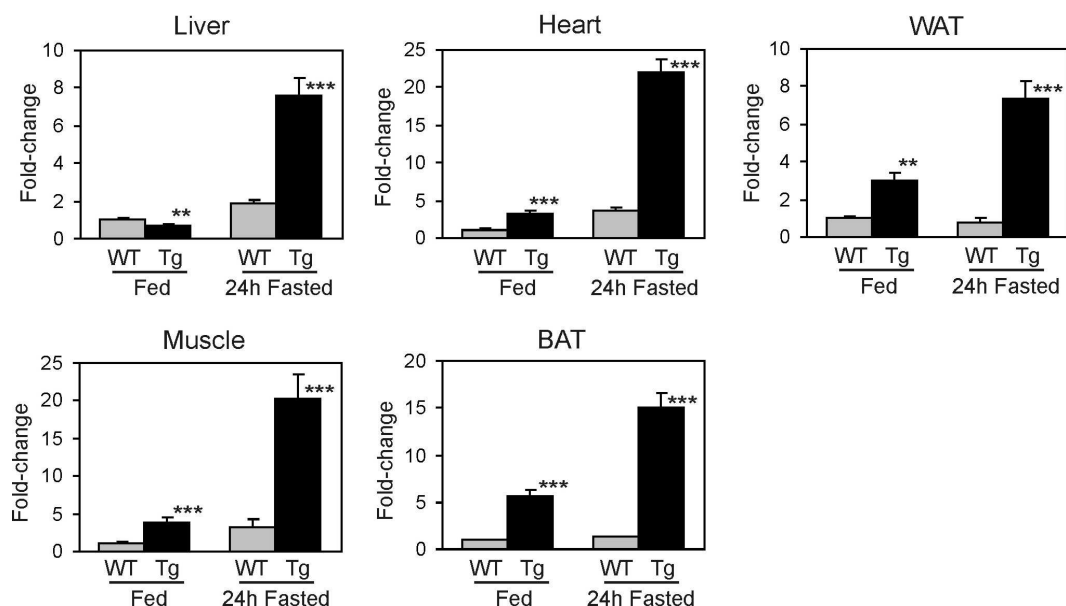
Supplemental Figure II: **No significant change in hepatic triglycerides in Angptl4-Tg mice.** Hepatic triglyceride concentration in fed and 24h fasted WT and Angptl4-Tg mice. Similar results were obtained by Oil red O staining of liver sections.
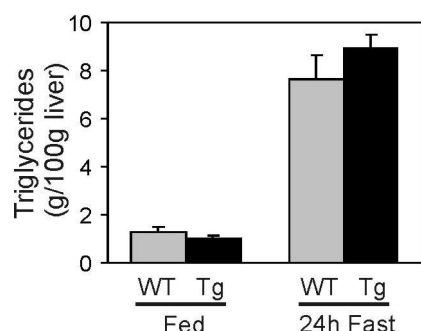
Supplemental Figure III: **Upregulation of genes involved in hepatic cholesterol synthesis in Angptl4-Tg mice.** Microarray analysis was performed on pooled livers (n=7) of 24h fasted WT and Angptl4-Tg mice. The threshold for fold-induction was set at 1.4. Approximately 40% of the probe sets upregulated in Angptl4-Tg mice represented genes involved in cholesterol synthesis or lipogenesis.

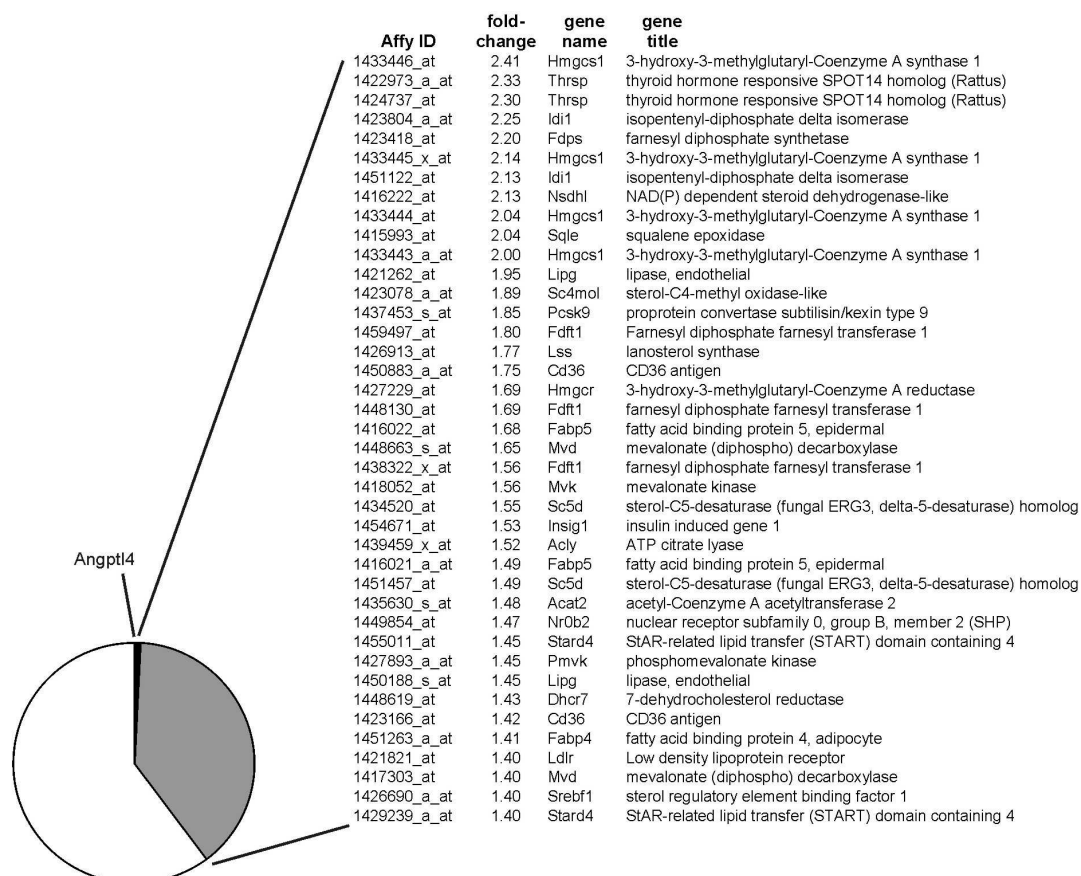| Affy ID	fold-change	gene name	gene title
1433446_at	2.41	Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
1422973_a_at	2.33	Thrsp	thyroid hormone responsive SPOT14 homolog (Rattus)
1424737_at	2.30	Thrsp	thyroid hormone responsive SPOT14 homolog (Rattus)
1423804_a_at	2.25	Idi1	isopentenyl-diphosphate delta isomerase
1423418_at	2.20	Fdps	farnesyl diphosphate synthetase
1433445_x_at	2.14	Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
1451122_at	2.13	Idi1	isopentenyl-diphosphate delta isomerase
1416222_at	2.13	Nsdhl	NAD(P) dependent steroid dehydrogenase-like
1433444_at	2.04	Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
1415993_at	2.04	Sqle	squalene epoxidase
1433443_a_at	2.00	Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
1421262_at	1.95	Lipg	lipase, endothelial
1423078_a_at	1.89	Sc4mol	sterol-C4-methyl oxidase-like
1437453_s_at	1.85	Pcsk9	proprotein convertase subtilisin/kexin type 9
1459497_at	1.80	Fdft1	Farnesyl diphosphate farnesyl transferase 1
1426913_at	1.77	Lss	lanosterol synthase
1450883_a_at	1.75	Cd36	CD36 antigen
1427229_at	1.69	Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
1448130_at	1.69	Fdft1	farnesyl diphosphate farnesyl transferase 1
1416022_at	1.68	Fabp5	fatty acid binding protein 5, epidermal
1448663_s_at	1.65	Mvd	mevalonate (diphospho) decarboxylase
1438322_x_at	1.56	Fdft1	farnesyl diphosphate farnesyl transferase 1
1418052_at	1.56	Mvk	mevalonate kinase
1434520_at	1.55	Sc5d	sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog
1454671_at	1.53	Insig1	insulin induced gene 1
1439459_x_at	1.52	Acly	ATP citrate lyase
1416021_a_at	1.49	Fabp5	fatty acid binding protein 5, epidermal
1451457_at	1.49	Sc5d	sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog
1435630_s_at	1.48	Acat2	acetyl-Coenzyme A acetyltransferase 2
1449854_at	1.47	Nr0b2	nuclear receptor subfamily 0, group B, member 2 (SHP)
1455011_at	1.45	Stard4	StAR-related lipid transfer (START) domain containing 4
1427893_a_at	1.45	Pmvk	phosphomevalonate kinase
1450188_s_at	1.45	Lipg	lipase, endothelial
1448619_at	1.43	Dhcr7	7-dehydrocholesterol reductase
1423166_at	1.42	Cd36	CD36 antigen
1451263_a_at	1.41	Fabp4	fatty acid binding protein 4, adipocyte
1421821_at	1.40	Ldlr	Low density lipoprotein receptor
1417303_at	1.40	Mvd	mevalonate (diphospho) decarboxylase
1426690_a_at	1.40	Srebf1	sterol regulatory element binding factor 1
1429239_a_at	1.40	Stard4	StAR-related lipid transfer (START) domain containing 4

Supplemental Figure IV: **Upregulation of genes involved in hepatic cholesterol synthesis in Angptl4-Tg mice.** mRNA expression of two genes involved in cholesterol synthesis was measured by real-time quantitative PCR. Errors bars represent SEM. n=7-10 mice/group. Statistical significance according to Student's t test. ***, p< 0.001.