ANGPTL8 requires ANGPTL3 to inhibit lipoprotein lipase and plasma triglyceride clearance

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Abstract Angiopoietin-like (ANGPTL)3 and ANGPTL8 are secreted proteins and inhibitors of LPL-mediated plasma triglyceride (TG) clearance. It is unclear how these two ANGPTL proteins interact to regulate LPL activity. ANGPTL3 inhibits LPL activity and increases serum TG independent of ANGPTL8. These effects are reversed with an ANGPTL3 blocking antibody. Here, we show that ANGPTL8, although it possesses a functional inhibitory motif, is inactive by itself and requires ANGPTL3 expression to inhibit LPL and increase plasma TG. Using a mutated form of ANGPTL3 that lacks LPL inhibitory activity, we demonstrate that ANGPTL3 activity is not required for its ability to activate ANGPTL8. Moreover, coexpression of ANGPTL3 and ANGPTL8 leads to a far more efficacious increase in TG in mice than ANGPTL3 alone, suggesting the major inhibitory activity of this complex derives from ANGPTL8. An antibody to the C terminus of ANGPTL8 reversed LPL inhibition by ANGPTL8 in the presence of ANGPTL3. The antibody did not disrupt the ANGPTL8:ANGPTL3 complex, but came in close proximity to the LPL inhibitory motif in the N terminus of ANGPTL8. Collectively, these data show that ANGPTL3 has a functional LPL inhibitory motif, but only inhibits LPL and increases plasma TG levels in mice in the presence of ANGPTL3.

MATERIALS AND METHODS

Mice

Angtlt3−/− and Angtlt8−/− mice were generated by homologous recombination using VelociGene technology as described previously (6, 7). Mice were housed (one to five per cage) in a controlled environment (12 h light/dark cycle, 22 ± 1°C, 60–70% humidity) and fed ad libitum with standard chow (Purina Laboratory Rodent Diet 5001, LabDiet). All animal procedures were conducted in compliance with protocols approved by the

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LPL is an enzyme important for clearance of triglycerides (TGs) from the circulation. The activity of LPL is tightly regulated through posttranslational mechanisms to accurately supply fatty acids to various tissues for storage or oxidation. It is well-established that angiopoietin-like (ANGPTL)3 and ANGPTL4 are negative regulators of LPL and that genetic inactivation of these secreted proteins reduces serum TG in mice and humans (1–3). ANGPTL8 also regulates TG metabolism and shares homology to the N-terminal portion of ANGPTL3 and ANGPTL4 (4–6). However, the exact mechanism by which ANGPTL8 modulates LPL activity and circulating TG remains to be established.

ANGPTL8 was reported to be a weak inhibitor of LPL activity (4). Hepatic overexpression of ANGPTL8 in WT mice is associated with hypertriglyceridemia, but no increase in plasma TG levels occurs when the same construct is expressed in Angtlt3−/− mice (5). Therefore, ANGPTL3 expression is required for ANGPTL8 to increase plasma TG levels. This contrasts ANGPTL3 (and ANGPTL4), which inhibits LPL independent of ANGPTL8. Here we provide evidence that ANGPTL3 induces a conformational change in ANGPTL8 that exposes its inhibitory motif to LPL.

Abbreviations: A3.mut, mutant angiopoietin-like 3; A8.mut, mutant angiopoietin-like 8; ANGPTL, angiopoietin-like; HDD, hydrodynamic DNA delivery; mAb, monoclonal antibody; RFU, relative fluorescence unit; TG, triglyceride; TR-FRET, time-resolved fluorescence energy transfer.

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**Plasmids**

A cDNA coding for a C-terminal V5 epitope-tagged human ANGPTL8 was generated as described elsewhere (6). The resulting human ANGPTL8/V5 cDNA was transferred from pRG977 into another mammalian expression vector, pRG1174 (CMV promoter). Full-length WT human ANGPTL3 was transfected with a heterologous signal sequence (mOR) and the same c-terminal tag (Myc.Myc.His6) was generated by PCR using a DNA plasmid clone (reference sequence NM_014495) as a template and the following primers: 5′-ATAACGTTCCTCAGCATTGAATGCAAGACATTG-3′ and 5′-TAGTAGTACCTCTCCTTCTCAAGCTTTTCTGAATGCTG-5′. The PCR product was cloned into direction Sphi cloning into a modified pRG977 expression vector. Human ANGPTL8 H40A/Q52A/H55A (mutant ANGPTL3 (A3.mut)) and human ANGPTL3 N48A/Q47A human ANGPTL8.V5 cDNA was transferred from pRG977 into another mammalian expression vector, pRG1174 (CMV promoter). Human ANGPTL8 H40A/Q52A/H55A (mutant ANGPTL3 (A3.mut)) and human ANGPTL8 (N48A/Q47A/H55A) [mutant ANGPTL3 (A3.mut)] mutations in the C-terminal Myc.Myc.His6 tag was generated by PCR using primers: 5′-GATCATCACCGAAGCCGTCATCCACG3′ and 5′-ATAGCTCCCGCTCCATATGGGAGCTGCTGCAAAGTTGGCG-3′.

**In vitro LPL inhibition assay**

Cells were transfected human ANGPTL3, human ANGPTL8, human LPL, or empty vector control, described above, using Mirus TransIT-LT1 transfection reagent (Mirus Bio). Antibodies were added 24 h posttransfection and incubated for another 24 h at 37°C. Then medium from cells transfected with human LPL was added and incubated for 6 h at 37°C. LPL activity was measured using EnzChek fluorogenic substrate assay (Molecular Probes) as described below. Alternatively, cell medium was collected at 72 h posttransfection, divided into different wells, and incubated with antibodies for 24 h. Then, medium from cells transfected with human LPL was added to each sample and lipase activity was measured with EnzChek fluorogenic substrate after a 6 h incubation at 37°C.

**EnzChek fluorogenic substrate lipase assay**

LPL activity was measured using the EnzChek fluorogenic substrate (9). Briefly, a 2× reaction mix was prepared freshly for each assay containing 4 mg/ml BSA, 0.025% Zwittergent-3-14 (Sigma-Aldrich), 7 μM EnzChek substrate (Molecular Probes), 200 nM APOC2 (Millipore) in phosphate saline buffer pH 7.5 with 1 mM CaCl2 and 0.5 mM MgCl2. The solution was mixed 1:1 with samples, fluorescence was measured every 30 s for 10 min at excitation 482 nm/emission 515 nm at room temperature. LPL activity was calculated from the linear slopes and expressed as RFUs.

**Epitope mapping**

Epitope mapping was performed by PEPSCAN PRESTO BE. Briefly, standard Fmoc-peptide synthesis was used to synthesize 15 amino acid-long peptides with 14 amino acid overlap. Anti-body binding to each peptide was tested in a PEPSCAN-based ELISA: primary antibody was incubated at 1 μg/ml (overnight at 4°C) followed by incubation with goat anti-human HRP conjugate (1 h at 25°C). After washing, the peroxidase substrate 2,2′-azino-di-3ethybenzthiazoline sulfonate and 10 μM/l of 3% H2O2 were added and color development was measured using a charge coupled device camera.

**AlphaLISA assay**

Assay was performed in a 384-well plate format at room temperature. CHO-K1 cell medium (2.5 μl) from cells transfected with a vector containing human ANGPTL3-myc and human ANGPTL8-V5 or empty vector was incubated with the indicated amounts of antibodies for 1 h in a final volume of 20 μl. Then anti-V5 Alpha acceptor beads (PerkinElmer) were added and incubated for an additional 30 min, followed by 1 h incubation with ...and streptavidin Alpha donor beads (PerkinElmer). All dilutions were performed in Hi-Block buffer (PerkinElmer), final concentration was 10 ng/ml.
for beads and 10 nM for biotinylated antibody in a total volume of 50 μl. AlphaLISA signal was measured in an Envision® multilabel reader (PerkinElmer) following the manufacturer’s instructions.

**TR-FRET antibody experiment**

HEK293T cells were cotransfected with a plasmid coding for ANGPTL3 and a plasmid coding for N-terminal Avi-tagged ANGPTL8 using TransIT-LT1 transfection reagent. Cell medium was collected after 72 h, concentrated 20× using Centriprep (Millipore) filter unit with a 10 kDa molecular cutoff, and site direct biotinylated using biotin-protein ligase BirA (Avidity) (10) following supplier’s instructions, then dialyzed against PBS. Biotinylation of Avi-ANGPTL8 was confirmed by Western blot using streptavidin-HRP for detection. Antibodies were labeled using Alexa FluorTM 647 antibody labeling kit (Invitrogen). Antibody and dye concentrations were determined spectrophotometrically and drug to antibody ratios of 5:2 were used for all experiments.

**TR-FRET was performed in a 384-well plate format.** Final concentration was 31.3 nM Europium-streptavidin, 25 nM labeled antibody, and 50% Biotin-Avi-ANGPTL8 in TR-FRET dilution buffer (PerkinElmer). TR-FRET was measured using Envision® multilabel reader (PerkinElmer): excitation filter, 340/30 nm; emission filter 1, 615/8.5 nm; emission filter 2, 665/7.5 nm; dichroic mirror, D400/D630; measured in a delay of 100 ms and a window time of 2 ms.

**Data analysis**

Data are expressed as mean ± SEM. Mean values were compared using two-way ANOVA as implemented in the GraphPad Prism 6.0 software (GraphPad Software, Inc.).

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**Fig. 1.** ANGPTL8 requires expression of ANGPTL3 to increase serum TG in mice. Serum TG (A) and Western blot analysis (B) of ANGPTL3 protein in plasma from Angptl8−/− mice 7 days after HDD-mediated expression of human ANGPTL3 or control vector (n = 5 per group). The + depicts ANGPTL3 control for Western blot. Serum TG (C) and Western blot detection (D) of ANGPTL8 in plasma from WT C57Bl6/J mice 7 days after HDD overexpression of human ANGPTL8 or control vector (n = 5 per group). The + depicts ANGPTL8 control for Western blot. Data are expressed as mean ± SEM. Statistical analysis was conducted by two-way ANOVA.

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**Fig. 2.** ANGPTL8 requires ANGPTL3 to inhibit LPL in vitro. A: HEK293T cells transfected with plasmids expressing human ANGPTL8, human ANGPTL3, or both of these two proteins were treated with control antibody or anti-ANGPTL3 blocking antibody for 24 h at 37°C and then incubated with medium from cells expressing human LPL for 6 h at 37°C. Lipase activity was measured as indicated in the Materials and Methods (n = 3, ****P < 0.0001 relative to control vector). B: Western blot detection of ANGPTL3 and ANGPTL8 in cell medium in the experiment described above. A3, ANGPTL3; A8, ANGPTL8; aA3 mAb, anti-ANGPTL3 blocking antibody; Control mAb, control antibody; IB, immunoblot. The experiment was repeated three times and the results were similar. Values are mean ± SEM. Statistical analysis was conducted by two-way ANOVA.
ANGPTL8 requires ANGPTL3 for its activity

To confirm the above findings, we transiently expressed human ANGPTL3 and human ANGPTL8 alone or in combination in HEK299T cells, which lack endogenous expression of ANGPTL3, ANGPTL8, and LPL. The transfected cells were treated with control antibody or anti-ANGPTL3 blocking antibody (11) and incubated with medium from cells transiently transfected with human LPL. The expression of ANGPTL8 alone, confirmed by Western blot, failed to inhibit LPL (Fig. 2A). However, expression of ANGPTL3 or coexpression of ANGPTL3 with ANGPTL8 strongly inhibited LPL activity (Fig. 2A). Interestingly, although the treatment with anti-ANGPTL3 antibody reversed ANGPTL3-induced inhibition of LPL, it failed to abolish the inhibition produced by coexpression of ANGPTL3 and ANGPTL8 (Fig. 2A). These results suggest that ANGPTL8 by itself does not inhibit LPL, but when coexpressed with ANGPTL3, it causes inhibition of LPL, an effect that cannot be reversed by an antibody to ANGPTL3.

ANGPTL8 contains functional LPL inhibitory motif

We next investigated whether the region on ANGPTL8 homologous to the inhibitory motifs described for ANGPTL3 and ANGPTL4 (8) could inhibit LPL (Fig. 3A). To this end, synthetic peptides containing the conserved inhibitory motif

![Inhibitory motif](image-url)

were designed and tested for their ability to inhibit LPL activity. As shown in Fig. 3B, the peptide containing the ANGPTL3 inhibitory motif (A3) showed an IC₅₀ of 5.7 µM, while the peptides containing the ANGPTL4 and ANGPTL8 inhibitory motifs (A4 and A8) showed IC₅₀ values of 2.9 µM and 4.1 µM, respectively. These results indicate that ANGPTL8 contains a functional LPL inhibitory motif, which is similar to those found in ANGPTL3 and ANGPTL4.

Fig. 3. ANGPTL8 sequence includes an active LPL inhibitory motif. A: Alignment of the sequences in the N-terminal region of human ANGPTL3, ANGPTL4, and ANGPTL8. Boxes indicate sequences of synthetic peptides used in the experiment. B: LPL activity measured in the presence of 20 nM bovine LPL and increasing concentrations of irrelevant peptide or peptides containing inhibitory motif of ANGPTL3, ANGPTL4, and ANGPTL8 after a 30 min incubation at room temperature. A3, ANGPTL3; A8, ANGPTL8; A4, ANGPTL4. The experiment was repeated three times and the results were similar.
Inhibitory motifs of ANGPTL3, ANGPTL4, and ANGPTL8 were incubated with bovine LPL. Although all three peptides inhibited the activity of LPL in a dose-dependent manner and with comparable potency, the efficacy was greatest for the peptide from the ANGPTL8 domain (Fig. 3B).

The ability of ANGPTL8 to block LPL does not require functional ANGPTL3 LPL inhibitory motif

To understand why the LPL inhibition produced by ANGPTL3 and ANGPTL8 coexpression was not blocked by an ANGPTL3 blocking antibody (see Fig. 2A), we modified the LPL inhibitory motifs of human ANGPTL3 and ANGPTL8 by site-directed mutagenesis. Three polar residues in the inhibitory motifs that are required for LPL inhibition (8) were replaced by alanine: N48A, Q53A, and H55A in ANGPTL3 and H40A, Q44A, and Q47A for ANGPTL8 (Fig. 4A). A8.mut expressed in the medium of HEK293T cells did not inhibit LPL. Coexpression of A8.mut with WT ANGPTL3 did not interfere with the ability of WT ANGPTL3 to inhibit LPL, and this effect was reversed when anti-ANGPTL3 antibody was added (Fig. 4B). When A3.mut was expressed alone, it failed to inhibit LPL. Coexpression of A3.mut with WT ANGPTL8 inhibited LPL activity, and this inhibition was not reversed by addition of the anti-ANGPTL3 blocking antibody (Fig. 4D). Both ANGPTL3 and ANGPTL8 proteins were readily detected by Western blot in the cell medium (Fig. 4C, E). These data suggest that the ANGPTL8 inhibitory motif is required for inhibition of LPL when ANGPTL8 is coexpressed with ANGPTL3.

Next we overexpressed the same constructs in the livers of WT mice. Expression of human mutated ANGPTL3 or ANGPTL8 did not increase plasma TG, showing that the proteins are inactive in vivo (Fig. 5A). Consistent with the observations in Angptl3−/− mice (see Fig. 1E), we observed a synergistic increase in serum TG in mice coexpressing ANGPTL3 and ANGPTL8 when compared with the effects of the individual proteins (Fig. 5A). The greater increase in serum TG when ANGPTL3 and ANGPTL8 were coexpressed occurred despite a much lower level of circulating ANGPTL3 (Fig. 5B).

When mutated ANGPTL3 was coexpressed with WT ANGPTL8, an increase in circulating TG of similar magnitude as that seen with coexpression of the WT proteins was seen (Fig. 5A). Conversely, coexpression of mutated ANGPTL8 and WT ANGPTL3 increased plasma TG to a level similar to that observed for WT ANGPTL3 alone. The levels of expression of ANGPTL3 and ANGPTL8 in the plasma are shown in Fig. 5B. These results confirm that...
ANGPTL3 and ANGPTL8 require functional LPL inhibitory motifs to mediate their effects in vivo.

Anti-ANGPTL8 blocking antibody reverses ANGPTL8-induced inhibition of LPL without disrupting the ANGPTL3 interaction

A library of peptides of 15 amino acids in length and with an offset of one residue was used to identify epitopes for eight monoclonal antibodies (mAbs) that bind human ANGPTL8 with high affinity (Kd = 2.4 × 10⁻¹⁰ M to 5.8 × 10⁻⁹ M; supplemental Fig. S1). The epitopes are depicted in Fig. 6A. The antibodies do not cross-react to mouse ANGPTL8 and were tested for in vivo efficacy in humanized ANGPTL8 mice (12). Surprisingly, only one antibody (mAb7 or REGN3776) produced a significant reduction in serum TG (Fig. 6B). This antibody binds to an epitope (amino acids 171–180) in the C-terminal region of ANGPTL8 (Fig. 6A). We have recently reported that this antibody lowers circulating TG by 65% in cynomolgus monkeys (12). Figure 6C shows that the antibody (EC₅₀ = 0.47 nM) reversed the inhibitory effect of ANGPTL8 on LPL in a dose-dependent fashion using medium from HEK293 cells coexpressing ANGPTL3 and ANGPTL8.

The anti-ANGPTL8 blocking antibody binds to the C-terminal region of ANGPTL8 and the LPL inhibitory motif is located in the N-terminal region (Fig. 6A). To test to determine whether mAb7 disrupted the interaction of ANGPTL3 and ANGPTL8, we used an AlphaLISA proximity assay. In this assay, a signal is detected when ANGPTL3 and ANGPTL8 are expressed together (Fig. 6D, left). The AlphaLISA signal remained strong even when high concentrations of the antibody were tested. These data suggest that the anti-ANGPTL8 blocking antibody does not restore LPL activity by disrupting the ANGPTL8:ANGPTL3 interaction.

Structural modeling has recently predicted that ANGPTL8 folds in a manner so that the N- and C-terminal domains are in close proximity (13). Thus, we hypothesized that the ANGPTL8 blocking antibody might sterically shield the inhibitory motif in ANGPTL8 to prevent LPL inhibition. To test this hypothesis, we labeled the ANGPTL8 blocking antibody and a nonblocking antibody to ANGPTL8 (mAb4), which bind to the mid region of ANGPTL8 protein sequence, with a FRET acceptor dye. In addition, we labeled ANGPTL8 in its N-terminal site with a donor dye, as depicted in Fig. 6E. When labeled ANGPTL8 was coexpressed with ANGPTL3 in HEK293 cells and assayed with the labeled antibodies, we detected a stronger TR-FRET signal with the ANGPTL8 blocking antibody directed to the C-terminal fragment compared with that of the nonblocking antibody (Fig. 6F). These results suggest that the N and C termini of ANGPTL8 are located in close proximity and that the ANGPTL8 blocking antibody may sterically prevent binding of the ANGPTL8 inhibitory motif to LPL.

DISCUSSION

In this study, we show that ANGPTL8 requires ANGPTL3 for its activity. We also reveal that ANGPTL8 has an inhibitory motif that is required for LPL inhibition when ANGPTL8 is coexpressed with ANGPTL3. In addition, we demonstrate that an anti-ANGPTL8 blocking antibody can revert the ANGPTL8:ANGPTL3-mediated inhibition of LPL by sterically preventing the accessibility of the ANGPTL8 inhibitory motif for LPL inhibition.

An interaction between ANGPTL8 and ANGPTL3 was suggested earlier. First, ANGPTL8 expression, which increases plasma TG levels in WT mice, fails to do so in Angptl3⁻/⁻ mice. Moreover, these secreted proteins coimmunoprecipitate from mouse serum after coexpression via recombinant adenovirus (5). It was suggested that ANGPTL8 activates ANGPTL5 by promoting its cleavage (5). However, later it was found that ANGPTL8 is not required for cleavage of ANGPTL3 (14). The present study used HDD to overexpress ANGPTL8 and ANGPTL3 in Angptl3⁻/⁻ and Angptl3⁻/⁻ mice to evaluate their relationship in vivo. Our results confirm the previous finding by Quagliarini et al. (5), demonstrating that ANGPTL8 can only increase serum TG in the presence of ANGPTL3. Nevertheless, we provided evidence that ANGPTL3 can modulate TG independently of ANGPTL8 and requires a functional LPL inhibitory motif for this activity. In parallel to the in vivo studies, we examined the effect of expression of ANGPTL3 and ANGPTL8 alone or in combination in culture cells on LPL activity. We confirmed that ANGPTL8 is inactive when expressed alone and found that a blocking antibody to ANGPTL3 did not reverse the inhibitory action of the ANGPTL3:ANGPTL8 complex on LPL activity.

Fig. 5. Coexpression ANGPTL8 with WT or mutated ANGPTL3 markedly increases serum TG in mice. A: C57Bl/6 mice were bled 7 days before (pre-bleed) and 7 days after the HDD of human WT or mutant forms of ANGPTL3 and ANGPTL8. B: Western blot analysis of plasma ANGPTL3 and ANGPTL8 for the experiment described in (A) (n = 4–5 mice per group). Statistics by two-way ANOVA analysis followed by a Bonferroni multiple comparison test are described in (A) (n = 4–5 mice per group). Statistics by two-way ANOVA analysis followed by a Bonferroni multiple comparison test are described in (A) (n = 4–5 mice per group).
These data demonstrate that the LPL suppressive activity of ANGPTL8 requires ANGPTL3.

The comparison of the region corresponding to the ANGPTL8 inhibitory motif to that of ANGPTL3 and ANGPTL8 deviates from the highly conserved consensus, LAXGLLXLGXGL (where X = polar residue), described for ANGPTL3 and ANGPTL4 (8) in three residues: position 2 (A→F), position 5 (L→F), and position 11 (G→L). We showed that these residues were not required for LPL inhibition. A synthetic peptide containing the ANGPTL8 inhibitory motif potently inhibited LPL. Two lines of evidence suggest that the LPL inhibitory motif on ANGPTL8

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**Fig. 6.** ANGPTL8 antibody reverses ANGPTL3:ANGPTL8-mediated LPL inhibition by steric interference of LPL with ANGPTL8 inhibitory motif. A: ANGPTL8 amino acid sequence depicting epitopes of eight mAbs raised against human ANGPTL8. B: Serum TGs of humanized ANGPTL8 mice, 7 days before (pre-bleed) and 2 days after a single subcutaneous injection of eight mAbs (10 mg/kg, n = 5 per group) with epitopes shown in (A). C: LPL activity in the medium of HEK293T cells expressing ANGPTL3 and ANGPTL8. The cell media were preincubated with mAb7 or control antibody for 24 h, followed by a 3 h incubation with media from cells expressing human LPL. D: AphaLISA assay to determine interaction between ANGPTL3 and ANGPTL8 coexpressed in CHO-K1 cells with no or increasing concentrations of either anti-ANGPTL8 blocking antibody (mAb7) or control antibody. E: Scheme of the TR-FRET assay with donor dye at the N terminus of ANGPTL8, near its inhibitory motif, and blocking and nonblocking antibodies labeled with acceptor dye. F: Energy transfer measured by TR-FRET assay between the N-terminus-labeled ANGPTL8 and control antibody, anti-ANGPTL8 blocking antibody (mAb7), or nonblocking anti-ANGPTL8 (mAb4) (n = 3; ***P < 0.0001). The experiment was repeated three times with the similar results. Bio, biotin; SA, streptavidin; D, donor dye; A, acceptor dye.
mediates the LPL inhibitory effect of the ANGPTL8:ANGPTL3 complex. First, an ANGPTL3 antibody that blocks the LPL activity of ANGPTL3 did not interfere with the activity of ANGPTL8:ANGPTL3. Second, the coexpression of inactive ANGPTL3 with WT ANGPTL8 in vivo resulted in a marked increase in serum TG.

The above findings suggest that ANGPTL8 exists in an inactive conformation where its inhibitory motif is not capable of inhibiting LPL (Fig. 7A). However, ANGPTL8 interaction with ANGPTL3 induces a conformational change that allows the inhibitory motif of ANGPTL8 access to LPL (Fig. 7B). Consistent with this scenario, incubation of ANGPTL8 with an antibody to the C-terminal domain reversed its inhibition of LPL activity (Fig. 7C). This observation suggests that the N and C termini of ANGPTL8 may be located in close proximity, which is consistent with a recent structural model proposed by Siddiqa et al. (13).

In summary, in vitro and in vivo studies in mice demonstrate that ANGPTL8 is inactive per se and requires ANGPTL3 for its activation and ability to inhibit LPL. This activation does not rely on ANGPTL3 activity. Our data suggest that the major LPL inhibitory activity derives from ANGPTL8 when both proteins are expressed together. This is supported by the observations that the ability of the ANGPTL8:ANGPTL3 complex to inhibit LPL could not be reversed by an anti-ANGPTL3 blocking antibody and depends on the active LPL inhibitory motif of ANGPTL8.

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