Pharmacological aspects of ANGPTL3 and ANGPTL4 inhibitors: New therapeutic approaches for the treatment of atherogenic dyslipidemia

# Manuscript Details

| | |
|---|---|
| **Manuscript number** | YPHRS_2019_2676_R1 |
| **Title** | Pharmacological aspects of ANGPTL3 and ANGPTL4 inhibitors: new therapeutic approaches for the treatment of atherogenic dyslipidemia |
| **Article type** | Review Article |


**Abstract**

Among the determinants of atherosclerotic cardiovascular disease (ASCVD), genetic and experimental evidence has provided data on a major role of angiopoietin-like proteins 3 and 4 (ANGPTL3 and ANGPTL4) in regulating the activity of lipoprotein lipase (LPL), antagonizing the hydrolysis of triglycerides (TG). Indeed, beyond low-density lipoprotein cholesterol (LDL-C), ASCVD risk is also dependent on a cluster of metabolic abnormalities characterized by elevated fasting and post-prandial levels of TG-rich lipoproteins and their remnants. In a head-to-head comparison between murine models for ANGPTL3 and ANGPTL4, the former was found to be a better pharmacological target for the treatment of hypertriglyceridemia. In humans, loss-of-function mutations of ANGPTL3 are associated with a marked reduction of plasma levels of VLDL, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Carriers of loss-of-function mutations of ANGPTL4 show instead lower TG-rich lipoproteins and a modest but significant increase of HDL. The relevance of ANGPTL3 and ANGPTL4 as new therapeutic targets is proven by the development of monoclonal antibodies or antisense oligonucleotides. Studies in animal models, including non-human primates, have demonstrated that short-term treatment with monoclonal antibodies against ANGPTL3 and ANGPTL4 induces activation of LPL and a marked reduction of plasma TG-rich-lipoproteins, apparently without any major side effects. Inhibition of both targets also partially reduces LDL-C, independent of the LDL receptor. Similar evidence has been observed with the antisense oligonucleotide ANGPTL3-LRX. The genetic studies have paved the way for the development of new ANGPTL3 and 4 antagonists for the treatment of atherogenic dyslipidemias. Conclusive data of phase 2 and 3 clinical trials are still needed in order to define their safety and efficacy profile.



| | |
|---|---|
| **Keywords** | angiopoietin-like 3 angiopoietin-like 4 antisense oligonucleotide evinacumab ANGPTL3-LRx |



| | |
|---|---|
| **Corresponding Author** | Massimiliano Ruscica |
| **Corresponding Author's Institution** | Università degli Studi di Milano |
| **Order of Authors** | Massimiliano Ruscica, Francesca Zimetti, Maria Pia Adorni, Cesare Sirtori, Maria Giovanna Lupo, Nicola Ferri |


| | |
|---|---|
| **Suggested reviewers** | Maciej Banach, Patrizia Tarugi, Amirhossein Sahebkar |

## Submission Files Included in this PDF

**File Name [File Type]**

C.L..docx [Cover Letter]

Response to the reviewers.docx [Response to Reviewers]

Graphical abstract REV.tif [Graphical Abstract]

ANGPTL review Pharmacol Res 2020 January 8th.docx [Manuscript File]

Figure 1b.tif [Figure]

Figure 2b.tif [Figure]

Conflict of interest.docx [Conflict of Interest]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

## Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
No data was used for the research described in the article

Dear Editor,

please find enclosed copy of the last version of the review article “Pharmacological aspects of ANGPTL3 and ANGPTL4 inhibitors: new therapeutic approaches for the treatment of atherogenic dyslipidemia” by Massimiliano Ruscica et al. to be considered for publication on Pharmacological Research.

We have answered all the issues have been raised. Moreover, we have added two tables and described the most recent study on ANGPTL3 antisense oligonucleotide (evinacumab) published few days ago on Journal of Lipid Research.

We are most grateful for your help and interest and we wish you a pleasant holiday time.

Massimiliano Ruscica

**Reviewer 1**

**This is an excellent, well written manuscript addressing a very timely and important subject. Bravo, my ongratulations to the authors.**

We really appreciated this comment. Thank you.

**Comments:**

**1. In the paragraph, lines 140-150, please rewrite to represent a summary of the current understanding of the mechanism of TG related risk:**

**a. Hepatic hypersecretion of TG enriched VLDL particles, VLDL itself being atherogenic,**

**b. Excess accumulation of remnant VLDL particles, especially emphasizing the type III dyslipiemia and role of RLP in general in hyperTG dyslipidemias in general,**

**c. Formation of small, dense LDL particles with discordantly high LDL particle concentrations for given LDL-C, Overall non-HDL particle load being the major mediator of risk.**

**d. reductions in HDL-C are largely driven by mass action exchange of cholesterol ester and TG between non-HDL and HDL particles via CETP, not a a causal mechanism of risk, shown not to be in the causal pathway by Mendelian Randomization experiments.**

We thank the reviewer for this constructive comment, we agree with. The text has been amended as follows "TG levels are the sum of the TG content in nascent very-low-density lipoproteins (VLDL) and in their remnants in the fasting state, together with TG in chylomicrons and their remnants in the postprandial state. Thus, TGRL remnants, encompassing a mixture of chylomicrons and VLDL particle, can be considered as a surrogate biomarker for plasma levels of both newly secreted TGRL and their remnants (7). As reported in the recent European Guidelines, desirable fasting TG levels are ≤ 150 mg/dL (1.7 mmol/L) and the use of drugs to lower TG should be considered in high-risk patients with TG > 200 mg/dL (2.3 mmol/L) and when TGs cannot be lowered by lifestyle changes (8). As TG levels rise over this range, large VLDL become the major TG-rich species due to either a rise in the hepatic production or a decrement in lipoprotein lipase (LPL) activity. In this scenario, LPL subsequently hydrolyzes VLDL to form smaller and denser lipoprotein particles, believed to be at least as atherogenic as LDL, being enriched in cholesteryl esters (9). Overall, with the exception of very large particles, e.g. chylomicrons, TGRL can enter the arterial wall contributing to the deposition of cholesterol content in the atherosclerotic plaque (10). In individuals with normal TG, for every chylomicron remnant particle there are approximately 10 VLDL particles, that have a short half-life compared to LDL. Considering that for every VLDL particle there are approximately 9 LDL particles, this proportion could explain why a rise in plasma TG will lead to many more LDL particles that VLDL particles (11). This ratio is not exactly maintained in type III hyperlipoproteinemia, a rarer phenotype characterized by abnormal apoB48 remnant particles plus abnormal VLDL apoB100 remnant particles, present at concentrations 30–50 times higher than normal remnant particles (12). The association between TG lowering and reduction of ASCVD risk has been recently reaffirmed in a meta-regression analysis of three classes of therapies (fibrates, niacin, and marine-derived omega-3 fatty acids) in addition to 25 statin trials, showing that TG lowering associates with a lower risk of major vascular events, even after adjustment for LDL-C lowering: Relative Risk (RR) 0.84; 95% CI, 0.75-0.94; per 1-mmol/L (40 mg/dL) TG reduction (13). Although similar conclusions have been reached by genetic studies supporting the causal role of raised TG levels on ASCVD risk (14, 15), it is worth mentioning that, in addition, the clinical benefit of lowering TG and LDL-C levels is proportional to the absolute change in apoB (16)."

**2. Lines 266-70, please expand discussion of mechanism of PPAR and interaction with ANGPTL providing greater detail for the reader to better frame the regulatory effects of PPAR at the nuclear level and the peripheral effects of ANGPTL 3/4/8 peripherally.**
Thank you. The text now reads as follows "ANGPTL4 expression is also induced by ligands of all peroxisome proliferator-activated receptors (PPAR-α, -δ, and γ) (77), although the fold induction of mRNA in tissues may vary, possibly due to individual variations among animal or in vitro models (78, 79)."

**3. Line 288: "...TR?? dependent manner..." is unclear**.
We thank the reviewer. The text has been amended and now reads as follows "Thyroid hormones suppress the gene expression of *ANGPTL3* but not that of *ANGPTL4* via activation of the thyroid hormone receptor β, thus providing a potential mechanism explaining the hypotriglyceridemic properties of thyroid hormone receptor β agonists (74). In patients with clinical and subclinical hypothyroidism, high ANGPTL3 levels have been described, an observation fitting with the negative correlation among ANGPTL3, total tri-iodothyronine and free tri-iodothyronine (81)."

**4. In conclusions section, it would be informative to the reader to briefly mention the problems that have arisen with both mAb or RNA interference strategies aimed at apoC3 suppression resulting in thrombocytopenia. Thus far, to my knowledge, this problem has not emerged as a problem with strategies using ANGPTL 3/4 as target of therapy.**
Thank you, this part has been added and the text now reads as follows "The ASO was safe, an important aspect, considering the thrombocytopenia found in patients given volanesorsen, an ASO against apoC-III , reducing chylomicron TG by roughly 83% (150)."

**Reviewer 2**
The manuscript of Ruscica and coll. is a revision regarding the pathophysiology and the emerging therapeutic approaches for the atherogenic dyslipidemia, a condition characterized by an abnormal increase of TG-rich lipoproteins (TRL). The work is interesting, well documented and written, focused on really new aspects of the metabolism of TRL that will be surely an important target for the development of future therapies in the cardiovascular field. I have only few comments, generally aimed to improving the fluidity and the clarity of the message. They are listed here below:

**Graphical abstract: the role of LPL should be better represented in the image, with the list of activator/inhibitors of the enzimatic activity. ANGPTL 3 and 4 should be graphically magnified, to stress the concept that they will be the main object of discussion.**
A modified graphical abstract has been added.

**Abstract: the first sentence (L 64-67) should focus on the residual risk in the statins era, i.e on the so-called "atherogenic dyslidemia" largely determined by a deranged TRL metabolism.** Thank you for this comment we agree with. The text now reads as follows "Among the determinants of atherosclerotic cardiovascular disease (ASCVD), genetic and experimental evidence has provided data on a major role of angiopoietin-like proteins 3 and 4 (ANGPTL3 and ANGPTL4) in regulating the activity of lipoprotein lipase (LPL), antagonizing the hydrolysis of triglycerides (TG). Indeed, beyond low-density lipoprotein cholesterol (LDL-C), ASCVD risk is also

dependent on a cluster of metabolic abnormalities characterized by elevated fasting and post-prandial levels of TG-rich lipoproteins and their remnants."

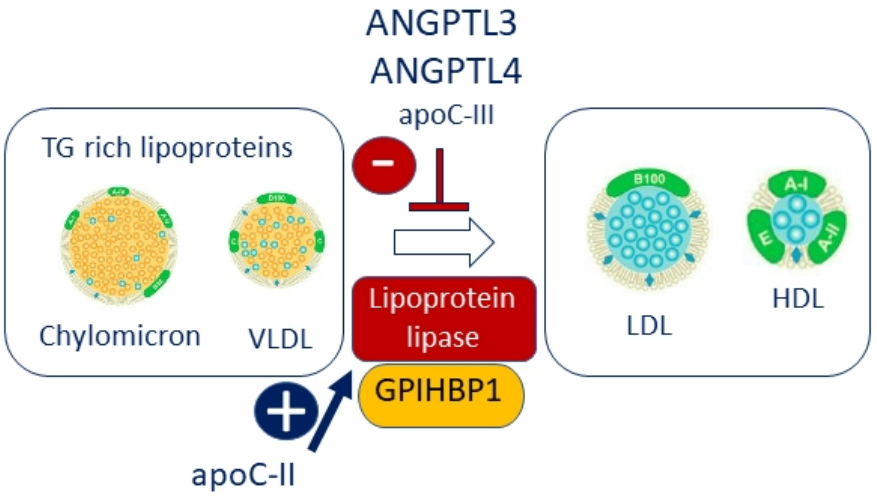
**L 78-79: the sentence may be shortened ("ANGPTL3 and ANGPTL4 are new therapeutic targets of biological therapies, *i.e.* monoclonal antibodies or antisense oligonucleotides").**
Thank you. The text has been amended *as per* suggestion.
**L 87: Please cut "thus suggesting their potential use in homozygous familial hypercholesterolemia", a comment that is not useful in Abstract but in Discussion .**
Thank you. The text has been amended *as per* suggestion

**Introduction: as above indicated, the relevance of the topic is largely dependent on the "residual risk after statins". The remaining text is indeed fully in line with this consideration. Part 3, L 281: what does it mean " Thyroid hormones .... to suppress ANGPTL3 mRNA in a TR??-dependent manner" In poarticular TR??- dependent manner? Please specify.**
Thank you. The text has been amended accordingly and now reads as follows "Thyroid hormones suppress the gene expression of *ANGPTL3* but not that of *ANGPTL4* via activation of the thyroid hormone receptor β, thus providing a potential mechanism explaining the hypotriglyceridemic properties of thyroid hormone receptor β agonists (74). In patients with clinical and subclinical hypothyroidism, high ANGPTL3 levels have been described, an observation fitting with the negative correlation among ANGPTL3, total tri-iodothyronine and free tri-iodothyronine (81)."

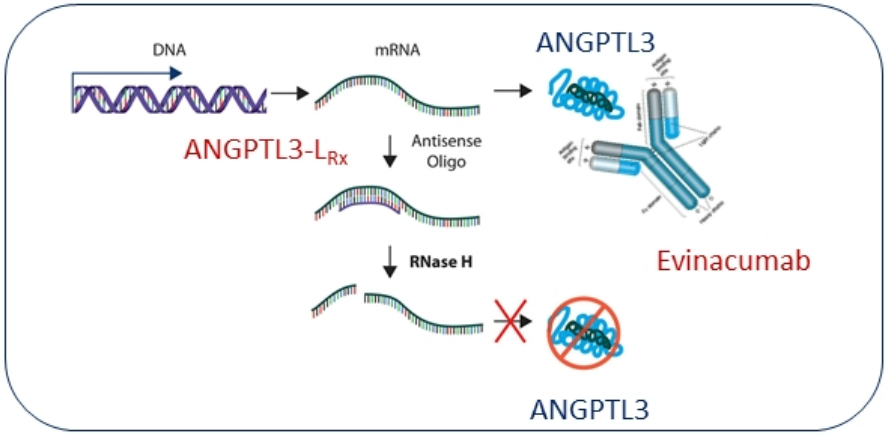
**In the same phrase the classification ( (i – ii- iii) does not appear logical; it may be better grouped in "This observation fits well (i) with the negative correlation between ANGPTL3, total tri-iodothyronine (TT3) and free tri-iodothyronine (FT3) (69) and (ii) with the high ANGPTL3 levels....."**
We thank the reviewer for this comment we agree with and the text has been amended accordingly.

**Part 4 , L 342: please write " in extenso" the acronymus BAT and WAT**
Thank you. BAT and WAT have been reported in extenso throughout the manuscript.

**PARt 7, L603: please specify that Fh is in the large majority of patients due to the loss of the LDL RECEPTOR..**
Thank you. The text has been amended accordingly and now reads as follows "Evinacumab was also tested in nine adults with homozygous familial hypercholesterolemia for LDLR, including two null homozygotes and one compound heterozygote with two null alleles."




## Pharmacological Inhibition of ANGPTL3



# Pharmacological aspects of ANGPTL3 and ANGPTL4 inhibitors: new therapeutic approaches for the treatment of atherogenic dyslipidemia

[1]Massimiliano Ruscica, [2]Francesca Zimetti, [2]Maria Pia Adorni, [3]Cesare R. Sirtori, [4]Maria Giovanna Lupo, [4]Nicola Ferri

[1]Dipartimento di Science Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy; [2] Dipartimento di Scienze degli Alimenti e del Farmaco, Università di Parma, Parma, Italy; [3] Dyslipidemia Center, A.S.S.T. Grande Ospedale Metropolitano Niguarda, Milan, Italy; [4]Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, Padua, Italy

Correspondence:

Massimiliano Ruscica

Università degli Studi di Milano

Via Balzaretti 9

20133 – Milan - Italy

e-mail: massimiliano.ruscica@unimi.it

tel: 0039-02-50318220

**Abstract**

Among the determinants of atherosclerotic cardiovascular disease (ASCVD), genetic and experimental evidence has provided data on a major role of angiopoietin-like proteins 3 and 4 (ANGPTL3 and ANGPTL4) in regulating the activity of lipoprotein lipase (LPL), antagonizing the hydrolysis of triglycerides (TG). Indeed, beyond low-density lipoprotein cholesterol (LDL-C), ASCVD risk is also dependent on a cluster of metabolic abnormalities characterized by elevated fasting and post-prandial levels of TG-rich lipoproteins and their remnants. In a head-to-head comparison between murine models for ANGPTL3 and ANGPTL4, the former was found to be a better pharmacological target for the treatment of hypertriglyceridemia. In humans, loss-of-function mutations of *ANGPTL3* are associated with a marked reduction of plasma levels of VLDL, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Carriers of loss-of-function mutations of *ANGPTL4* show instead lower TG-rich lipoproteins and a modest but significant increase of HDL. The relevance of ANGPTL3 and ANGPTL4 as new therapeutic targets is proven by the development of monoclonal antibodies or antisense oligonucleotides. Studies in animal models, including non-human primates, have demonstrated that short-term treatment with monoclonal antibodies against ANGPTL3 and ANGPTL4 induces activation of LPL and a marked reduction of plasma TG-rich-lipoproteins, apparently without any major side effects. Inhibition of both targets also partially reduces LDL-C, independent of the LDL receptor. Similar evidence has been observed with the antisense oligonucleotide ANGPTL3-$L_{RX}$. The genetic studies have paved the way for the development of new ANGPTL3 and 4 antagonists for the treatment of atherogenic dyslipidemias. Conclusive data of phase 2 and 3 clinical trials are still needed in order to define their safety and efficacy profile.

**Keywords**: angiopoietin-like 3, angiopoietin-like 4, antisense oligonucleotide, evinacumab, ANGPTL3-$L_{Rx}$

## 1. Introduction

A series of modifiable and non-modifiable risk factors contributes to the atherosclerotic cardiovascular disease (ASCVD). Among them, the pharmacological control of hypercholesterolemia has represented the most effective therapy in the prevention of CVD. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, statins, successfully lower low-density lipoprotein cholesterol (LDL-C) and reduce the rate of CV events in many patients. Despite the fact that LDL-C is causal in the development of atherogenesis and ASVCD, new options are required to control high levels of triglyceride-rich lipoproteins (TGRL) (1) or lipoprotein (a)(2). Beyond LDL-C, ASCVD risk is also dependent on a cluster of metabolic abnormalities characterized by elevated fasting and post-prandial levels of TGRL and their remnants (3), low levels of high-density lipoprotein cholesterol (HDL) and elevated small dense LDL (4). Thus, besides therapies directed at reducing only LDL-C levels, pharmacological approaches targeting other lipoproteins, *e.g.* triglycerides (TG) and/or HDL-C (5), can be considered in order to reduce the CV residual risk (6).

TG levels are the sum of the TG content in nascent very-low-density lipoproteins (VLDL) and in their remnants in the fasting state, together with TG in chylomicrons and their remnants in the postprandial state. Thus, TGRL remnants, encompassing a mixture of chylomicrons and VLDL particle, can be considered as a surrogate biomarker for plasma levels of both newly secreted TGRL and their remnants (7). As reported in the recent European Guidelines, desirable fasting TG levels are ≤ 150 mg/dL (1.7 mmol/L) and the use of drugs to lower TG should be considered in high-risk patients with TG > 200 mg/dL (2.3 mmol/L) and when TGs cannot be lowered by lifestyle changes (8). As TG levels rise over this range, large VLDL become the major TG-rich species due to either a rise in the hepatic production or a decrement in lipoprotein lipase (LPL) activity. In this scenario, LPL subsequently hydrolyzes VLDL to form smaller and denser lipoprotein particles, believed to be at least as atherogenic as LDL, being enriched in cholesteryl esters (9). Overall, with the exception of very large particles, *e.g.* chylomicrons, TGRL can enter the arterial wall contributing to the deposition of cholesterol content in the atherosclerotic plaque (10). In individuals with normal TG, for every chylomicron remnant particle there are approximately 10 VLDL particles, that have a short half-life compared to LDL. Considering that for every VLDL particle there are approximately 9 LDL particles, this proportion could explain why a rise in plasma TG will lead to many more LDL particles that VLDL particles (11). This ratio is not exactly maintained in type III hyperlipoproteinemia, a rarer phenotype characterized by abnormal apoB48 remnant particles plus abnormal VLDL apoB100 remnant particles, present at concentrations 30–50 times higher than normal remnant particles (12).

The association between TG lowering and reduction of ASCVD risk has been recently reaffirmed in a meta-regression analysis of three classes of therapies (fibrates, niacin, and marine-derived omega-3 fatty acids) in addition to 25 statin trials, showing that TG lowering associates with a lower risk of major vascular events, even after adjustment for LDL-C lowering: Relative Risk (RR) 0.84; 95% CI, 0.75-0.94; per 1-mmol/L (40 mg/dL) TG reduction (13). Although similar conclusions have been reached by genetic studies supporting

the causal role of raised TG levels on ASCVD risk (14, 15), it is worth mentioning that, in addition, the clinical benefit of lowering TG and LDL-C levels is proportional to the absolute change in apoB (16).

Catalyzing the partial hydrolysis of the core TG of circulating chylomicrons and VLDL to non-esterified fatty acids and 2-monoacylglycerol for tissue utilization, LPL represents the ideal target to control hypertriglyceridemia (17). TGRL are too large to cross capillary endothelia and thus, in order to gain access to triacylglycerols in these particles, LPL must be exposed to the luminal surface of the capillary endothelial cells. Once produced and correctly folded in myocytes and adipocytes, LPL is secreted into the subendothelial space and translocated to the lumen of capillaries by the glycosyphosphatidylinositol-anchored HDL binding protein (GPIHBP1) (18, 19), that retains LPL anchored to the endothelium (20-23). Highly charged, membrane bound chains of heparan sulphate-proteoglycans (HSPG) also contribute to anchor LPL on the luminal surface (17, 24), but since this binding is weak, LPL is shuttled to GPIHBP1 (25): the enzymatically active form of LPL is a 1:1 heterodimeric complex with GPIHBP1 (26, 27). Nevertheless, GIPHBP1 stabilizes LPL, thus enhancing its lipase activity (28, 29), an effect confirmed in GPIHBP1 knockout mice and patients with loss-of-function mutations of *GPIHBP1* which associated with hyperchylomicronemia (21, 23). GPIHBP1 is expressed highly in heart, adipose tissue and skeletal muscle, the same tissues that express high levels of LpL. In each of these tissues, GPIHBP1 is located on the luminal face of the capillary endothelial cells and not in the endothelium of brain capillaries (18, 21). This suggests a more important role for HSPG in tethering LPL to atherosclerosis-prone vessels (30). The identification of autoantibodies against GP1HBP1 has explained the reason why patients who have a clinical manifestation of familial chylomicronemia syndrome (about 3%), are negative to the genetic screening of five canonical genes for monogenic chylomicronemia (31, 32).

Rare loss-of-function mutations of genes involved in TG metabolism, such as *LPL* (33, 34), are associated to higher plasma TG levels and CV risk, whereas genetic inactivation of *ApoC3*, *Angptl3* and *Angptl4* genes, which encode for natural inhibitors of LPL, are associated with lower TG levels and a corresponding lower CVD risk (35-37). Importantly, some variants of *LPL* and *LDL receptor* (*LDLR*) are associated to similar lower coronary heart disease (CHD) risk per unit and to lower levels of apoB-containing lipoproteins (16). This association is additive and proportional to the absolute change in apo-B, indicating that the clinical benefit of TG lowering is similar to the clinical benefit of LDL-C lowering (16). Based on these findings, several novel therapies that potentially reduce TGRL are currently in development (38-40). Although many pharmacological targets can be envisioned to reduce TGRL levels, lipase antagonism may represent a successful example of clinical relevance (41).

In this complex scenario, another main player is represented by the apolipoprotein (apo)C-II. Synthetized in the liver and intestine, it is secreted as a surface component of chylomicrons, VLDL and HDL representing a specific activator/cofactor of LPL (42). The presence of apoC-II is required for a proper enzymatic activity of LPL, whereas three proteins from the angiopoietin-like (ANGPTL) family - ANGPTL3, ANGPTL4 and ANGPTL8 (37, 38, 43-45) as well as apoC-III are its physiological inhibitors (46). Thus, the

present review will discuss the current development of new pharmacological inhibitors of ANGPTL3 and ANGPTL4 as potential treatments for treating hypertriglyceridemia and associated CVD risk.

## 2. Biology of AGPTL3, ANGPTL4 and ANGPTL8

Angiopoietins are members of the vascular endothelial growth factor (VEGF) family (47-49). ANGPTL3 is a 70kDa protein mainly expressed and secreted by the liver (43, 50), and to a lesser extent by the kidney (50), from which it is released at higher levels in case of renal damage (51-53). ANGPTL3 shares with its family members a N-terminal and a C-terminal fibrinogen-like domain (FLD), conserving the fibrinogen domain (~40% of sequence identity), except for a signal peptide (16 amino acids) required for its secretion (54, 55) (Figure 1). A linker region between N- and C-terminal domains is necessary for the ANGPTL3 biological activation. After intracellular and extracellular cleavages by furin (PCSK3) and PACE4 (PCSK6), respectively (56), the N-terminal domain is released being able to inhibit the activity of LPL more efficiently than full-length ANGPTL3 (54, 57): ANGPTL3 circulates in the plasma as full-length and truncated forms.

The human ANGPTL4 glycoprotein (~45-65 KDa) contains an N-terminal coiled-coil structure and a C-terminal FLD (58) (Figure 1). ANGPTL4 shares with ANGPTL3 31% of the amino acid sequence identity as well as modular structure. ANGPTL4, discovered independently during a search of additional angiopoietin-related proteins (59), is induced by fasting (60) and during preadipocyte differentiation (61). ANGPTL4 is produced in many cells and tissues, including adipose tissue, liver, intestine and muscle (61). Before secretion ANGPTL4 forms dimers and tetramers, whereas after secretion it undergoes cleavage at a canonical proprotein convertase cleavage site, *i.e.*, 161-Arg-Arg-Lys-Arg-164 (62). After cleavage, the N-terminal fragment, which remains oligomerized, is allowed to bind transiently to LPL. This process converts LPL from catalytically active dimers to inactive monomers, thus reducing the LPL activity (63).

Differently from the above described members of the same family, ANGPTL8 (also known as betatrophin) lacks the FLD leading to a protein of 22 kDa, *i.e.* less than half the size of ANGPTL3 and ANGPTL4 (45, 64) (Figure 1). The comparison between the ANGPTL8 sequence and the N-terminal domains of ANGPTL3 and ANGPTL4 highlighted the presence of a Specific Epitope1 (SE1) region, also mapped on ANGPTL3 and ANGPTL4, as necessary and sufficient to inhibit LPL activity (65-67). ANGPTL8 is a feeding-induced hepatokine, highly expressed in liver, white adipose tissue and brown adipose tissue (45, 68, 69).

## 3. Transcriptional regulation of ANGPTL3 and ANGPTL4

*ANGPTL3* is transcriptionally regulated by Liver X receptors (LXRs) and Hepatocyte Nuclear Factor 1α (HNF1α) (70). This evidence derived from mice fed to a high-cholesterol diet and treated with a synthetic LXR-selective agonist (T0901317); this raises the hepatic gene expression of *Angptl3* (70). The analysis of the transcriptional response elements of mouse and human ANGPTL3 genes identified an LXR binding site, required for the regulation by LXR transcription factors (70, 71). LXR also drives the expression of LPL (72)

and ANGPTL8 (73), thus suggesting the presence of a common regulatory pathway. In contrast, HNF1α indirectly inhibits the expression of ANGPTL3, *i.e.* through the activation of the thyroid hormone receptor (74). A repressive stimulus on the transcriptional activity of ANGPTL3 is mediated by insulin and leptin, thus supporting the hypothesis of a possible link between diabetic condition and dyslipidemia (75, 76). In adipose tissue and liver, *ANGPTL4* expression is regulated by feeding, fasting, with a strong induction under this latter condition, suggesting a pivotal role in fat metabolism (60). *ANGPTL4* expression is also induced by ligands of all peroxisome proliferator-activated receptors (PPAR-α, -δ, and γ) (77), although the fold induction of mRNA in tissues may vary, possibly due to individual variations among animal or *in vitro* models (78, 79).

A head-to-head comparison between ANGPTL3 and ANGPTL4 clearly pointed out that, although both inhibit LPL activity and raise plasma TG levels, they are regulated by different nuclear receptors (78). In particular, ANGPTL4 expression is induced in many tissues, including heart and skeletal muscle, whereas LPL hydrolyzes circulating lipoproteins for energy expenditure (78). Consistently, ANGPTL4 overexpression in the heart reduces the use of lipoprotein-derived free fatty-acids (FFA) in cardiac tissue, a mechanism mediated by a repression of the LPL activity (80).

Thyroid hormones suppress the gene expression of *ANGPTL3* but not that of *ANGPTL4* via activation of the thyroid hormone receptor β, thus providing a potential mechanism explaining the hypotriglyceridemic properties of thyroid hormone receptor β agonists (74). In patients with clinical and subclinical hypothyroidism, high ANGPTL3 levels have been described, an observation fitting with the negative correlation among ANGPTL3, total tri-iodothyronine and free tri-iodothyronine (81).

**4. Role of ANGPTL3 and ANGPTL4 on lipid metabolism**

Both ANGPTL3 and ANGPTL4 are involved in the regulation of breakdown and lipid storage. ANGPTL3 decreases VLDL-TG clearance by different mechanisms: by inhibiting LPL activity (82) and by a direct activation of lipolysis in adipocytes (83), a process resulting in FFA and glycerol release into the circulation (83). LPL is involved in lipid-related pathological conditions, including atherosclerosis (84), diabetes and obesity, Alzheimer's disease and cachexia (17). Beyond LPL, evidence coming from experimental models suggested an inhibitory effect of ANGPTL3 on endothelial lipase (EL) (85). This enzyme is expressed by endothelial cells and acts in the plasma similar to LPL (85, 86).

Since the N-terminal domain of ANGPTL3 interacts directly with LPL (65) and EL (85), it is not surprising that the presence of this domain is required for the inhibitory activity (54). This feature is completely abolished in the absence of the heparan sulphate-proteoglycans (HSPG) that anchor EL to endothelial cells (85). However, the molecular mechanism underlying the inhibition of LPL by ANGPTL3 is still unclear. It has been hypothesized that ANGPTL3 may induce LPL cleavage by the way of proprotein convertases PACE4 and furin, an effect specific for LPL but not for EL (87). Overall, the mechanism of ANGPTL3 is thus to foster TG to adipose tissue for storage during feeding through the tissue specific expression of the

modulator ANGPTL8 (64). Regarding ANGPTL8, its inhibitory activity on LPL is observed only in the presence of ANGPTL3 (88-90). Beyond LPL, ANGPTL3 inhibits EL, a crucial enzyme regulating plasma HDL levels (91). In humans, ANGPTL3 concentration positively correlated with HDL-C (85), a finding dependent on the inhibition of EL activity.

All-in-all, it is possible to envision that ANGPTL3, 4 and 8 regulate TG metabolism by inhibiting LPL in different tissues and under different nutritional status. During fasting an induction of ANGPTL4 and a suppression of ANGPTL8 (92-94) were observed, a condition affecting ANGPTL3 activity. The suppression of ANGPTL8 during fasting state is mediated by the increased levels of glucocorticoids and their binding to negative glucocorticoid responsive elements in the promoter region (94). Downregulation of ANGPTL8 leads to a higher activity of LPL in the skeletal muscle and heart (95), thus promoting TG hydrolysis and increased circulating FFA concentrations. Conversely, increased ANGPTL4 protein reduces LPL activity in the adipose tissue (96), promoting lipolysis in the adipocytes (97). This site-specific modulation of LPL activity leads the circulating TG toward peripheral tissues for utilization. Conversely, feeding leads to the upregulation of ANGPTL8 and the downregulation of ANGPTL4, directing plasma TG to adipose tissues for storage. Apart from fasting, during physical exercise, ANGPTL4 is induced in non-exercising skeletal muscle, likely serving to divert plasma TG to exercising muscle to be used as energy source (98). In addition, during cold exposure, ANGPTL4 is suppressed in brown adipose tissue and induced in white adipose tissue, thus ensuring an adequate energetic provision of TG to brown fat cells (96, 99). Overall, ANGPTL4 is to be considered as part of a shuttling mechanism directing fatty acids derived from circulating TGRL to brown adipose tissue during sustained cold exposure (96).

**5. Phenotype of *Angptl3* and *Angptl4* knock-out and transgenic mice**

The first genetic evidence of the role of ANGPTL3 on lipid metabolism was made by Koishi *et al* who identified an insertion mutation of the *Angptl3* gene associated with a hypolipidemic phenotype in obese KK mice (100). Levels of TG, total cholesterol and free fatty acids (FFA) in these mice were lower than those of wild type mice. Overexpression of *Angptl3* in murine models, *e.g.* by injection of adenovirus or human ANGPTL3 or administration of recombinant ANGPTL3, led to significant increases of TG, total cholesterol and FFA levels within one day post injection, reaching a peak after about four days (100). Generation of *Angptl3* knock-out mice corroborated its role in lipid metabolism (101). The absence of ANGPTL3 reduces TG, total cholesterol and FFA concentrations with a concomitant rise in LPL activity, *i.e.* +1.57 fold compared to wild type mice (101). In addition, the *Angptl3*-null mice fed a high fat diet had lower adipose tissue weight despite no differences in adipocyte size (101). The same experimental model showed a significant fall in the uptake of circulating VLDL-TG into white adipose tissue, rather than into skeletal muscle, brow adipose tissue and heart (102). The effect on the adipose tissue has been further corroborated by observations that in the absence of ANGPTL3 or ANGPTL8 fat mass is reduced after a +1°C increase in temperature in the fed (not fasted)

condition, without any change in physical activity or food intake (103). In addition, short-term cooling was shown to increase plasma ANGPTL4, ANGPTL3 and ANGPTL8 levels in young, healthy, lean men (104). These ANGPTLs are thought to act in concert to facilitate TG partitioning among tissues in response to cold (104).

Thus, ANGPTL3 or ANGPTL8 are essential for an efficient storage of dietary TG and deletion of these genes increases energy use and feeding-induced thermogenesis (103). In fed mice, the pharmacological inactivation of ANGPTL3 by the use of antibodies leads to a reduced hepatic secretion and plasma levels of TG (105). Besides TG, ANGPTL3 inactivation reduced LDL-C and apoB levels, despite no changes in hepatic apoB secretion, thus suggesting an increased clearance of apoB-containing lipoproteins (105). Similar lipid-modifying effects have been described, in a model of dyslipidemic Cynomolgus monkeys, after the administration of an antibody against ANGPTL3 (106).

Considering the ability of ANGPTL4 to inhibit LPL, knock-out mice showed a decrement of plasma TG and a faster initial weight gain when these were fed with a HFD (107, 108). Unexpectedly, the growth of *Angptl4*-null mice reached a plateau after 12 weeks of age, after that an opposite effect was seen, *i.e.* weight loss associated to an anorexic state. This condition led to a premature death between weeks 15 and 25 due to the development of severe fibrinopurulent peritonitis with ascites (107). In the same model, an intestinal fibrosis was observed, with a compressed liver and a hyperplastic spleen. Moreover, mesenteric lymph nodes underwent dramatic expansion and contained numerous lipid-laden macrophages (107).

The regulation of LPL by ANGPTL4 is essential for the protection from the proinflammatory effects induced by saturated fatty acids, leading to lipid accumulation into macrophages within mesenteric lymph nodes. This effect is associated to foam cell formation and a massive inflammatory response characterized by severe mesenteric lymphadenitis. These data suggest that the homozygous carrier status of the E40K mutation in *Angptl4*, associated to higher LPL activity and lower plasma TG (62, 109), may be more sensitive to the proinflammatory effects of dietary saturated fat. Even more intriguingly, recent evidence highlighted a relevant anti-inflammatory action of ANGPTL4, exerted by modulating macrophage polarization by mesenchymal stem cells during cardiac repair (110).

Finally, relative to glucose metabolism findings are not conclusive. In transgenic mice overexpressing *Angptl4* it has been reported (i) no impairment on glucose levels, (ii) a rise in blood glucose levels (44, 111), and (iii) an improvement or an impairment in glucose tolerance (112-114). In contrast, the functional studies on *Angptl4*-null mice demonstrated an improvement in insulin sensitivity and glucose homeostasis (115), suggesting that the inhibition of ANGPTL4 may reduce the risk of type 2 diabetes.

**6. Preclinical development of ANGPTL3 and ANGPTL4 inhibitors**

The identification of individuals carrying rare loss-of-function variants in *Angptl3* has confirmed the relevant role of this protein in the metabolism of TGRL, also considering that these subjects have lower TG levels, LDL-C and HDL-C (43, 116-119). This lipid profile is defined familial combined hypolipidemia and it seems not

associated with peculiar pathological manifestations (120). Similar findings in the lipid profile were described for variants of ANGPTL4, although these subjects had reduced TG but elevated HDL-C (78).

Interestingly, the use of a humanized mouse model deficient in the *Ldlr* highlighted that silencing of ANGPTL3 led to a modest reduction on LDL-C, thus suggesting that the LDL-C lowering was linked to *Ldlr* (92). This observation has been supported in *Ldlr*$^{+/-}$ mice injected with silencing *Angptl3* and PCSK9. Reduction of total cholesterol (TC) and LDL-C levels were larger than those achieved from silencing *Angptl3* or *PCSK9* alone (121). Currently, an open question is how inactivation of ANGPTL3 leads to the reduction in LDL-C levels. Some hypotheses have been raised: (i) changes in VLDL apoB production rate or LDL apoB fractional catabolic rate (86), (ii) reduced apoB secretion and enhanced uptake of apoB-containing lipoproteins (93), and (iii) increased LDL clearance due to a rise in the inactive form of PCSK9 (94). On the other hand, the raising effect of ANGPTL3 on HDL seems to be the direct consequence of the inhibitory phospholipase activity on EL (122).

A role of ANGPTL3 and ANGPTL4 has also been documented with respect to the HDL promoting-cholesterol efflux. A direct correlation has been in part observed between plasma levels of these proteins and the HDL cholesterol efflux capacity in humans (123-125). Overall, the plasma concentrations of HDL of the studied subjects mainly accounted for the variation observed in HDL cholesterol efflux capacity.

Genetic variants leading to reduction in the levels of ANGPTL3 and ANGPTL4 are associated with a reduced CHD risk, with odds ratios ranging between 0.61 and 0.66 (11, 12, 14). In particular, null variants of ANGPTL3 lead to a unique form of familial hypobetalipoproteinemia, characterized by lower levels of all lipoproteins (116) and enhanced insulin sensitivity without an increased prevalence of fatty liver disease (116).

The explanation for a reduced CVD risk may be related to a life-long exposure to low levels of LDL-C. However, the extent of risk reduction in ANGPTL3 loss-of-function variants is estimated to be larger than the one predicted by the LDL lowering effect, suggesting that the stimulation of LPL-promoted lipolysis might translate into additional cardiovascular protection (126). These genetic observations strongly support the utility of developing new ANGPTL3 and 4 inhibitors to reduce TG and the incidence of CVD. A summary of the pre-clinical evidence relative to ANGPTL3 or ANGPTL4 inhibitors is shown in Table 1A.

Evinacumab (REGN1500) is a fully human monoclonal antibody directed to ANGPTL3 (Box1)(127). By using surface plasmon resonance, Gusarova et *al.* measured the relative affinity of evinacumab to human, monkey, rat and mouse ANGPTL3 (106) and showed that the drug binds ANGPTL3 with comparable affinities in all four species (Kd=0.26÷1.28 nM). In addition, evinacumab reverted the inhibition of LPL mediated by ANGPTL3 with $IC_{50}$ values of 2.9÷9.6 nM (106). In an experimental model of hypercholesterolemic mice, the injection of 25 mg/kg once weekly of evinacumab for 8 weeks dramatically reduced TG (-53%), total cholesterol (-35%) and LDL-C (-45%) (106). These findings were then reproduced in nonhuman primates (Cynomolgus monkeys) treated with single doses of evinacumab (106). The 3 mg/kg dose reduced by 48% plasma TG levels, with a doubling of effect with 10 mg/kg (-89%). The lipid lowering effect was maintained

for 33 days after the single injection (106). Very recently, in APOE*3-Leiden.CETP mice, triple treatment with atorvastatin + alirocumab + evinacumab was superior to atorvastatin in reducing plasma total cholesterol (-68%), non-HDL-C (-84%), and TG levels (-67%). Relative to atheroma formation and composition, the effect of triple combo blocked progression and led to a stronger regression of atherosclerotic lesion size, resulting in a further -56% macrophage content compared with control, in parallel with a rise in α-smooth muscle cells and collagen content (128).

In addition to evinacumab, antisense oligonucleotides (ASOs) targeting ANGPTL3 messenger RNA are under clinical evaluation (Figure 2). ANGPTL3$_{Rx}$ is a second-generation 2′-O-methoxyethyl (2'-MOE) chimeric antisense oligonucleotide targeted to ANGPTL3 mRNA consisting of the nucleotide sequence 5'-GGACATTGCCAGTAATCGCA-3'. ANGPTL3-L$_{Rx}$ is a second-generation ASO drug targeting ANGPTL3 mRNA with the same sequence and sugar modifications as ANGPTL3$_{Rx}$ but the addition of a covalent linkage with a triantennary N-acetyl galactosamine (GalNAc) cluster, conferring high affinity for the hepatocyte-specific asialoglycoprotein receptor (ASGPR)(129) (Box2). The GalNAc cluster enhances delivery of ANGPTL3-L$_{Rx}$ to hepatocytes over other cell types and consequently increases drug potency for targets expressed by these cells (130).

Mouse *Angptl3* ASO has been tested in different animal models, including the *Ldlr*$^{-/-}$ and Apoc3$^{-/-}$ mice, and mice expressing human apoC-III, either fed with chow diet or Western diet (131). Administration of the murine *Angptl3* ASOs led to a drop in *Angptl3* mRNA expression between 69 and 91% corresponding to a decrement in protein levels of 50-90% in each of these mouse models. Relative to the lipid profile, TG, LDL-C and HDL-C were all reduced, *i.e.* between 35-85%, 7-64% and 3-23%, respectively (131). These findings show that the TG and LDL-C lowering driven by the silencing of ANGPTL3 is independent of the LDLR pathway and occurs in the absence or presence of an excess of apolipoprotein C-III which exhibits inhibitory LPL activity (46).

Experiments on the *Ldlr*$^{-/-}$ model allowed to verify that upon a Western-diet administration, the *Angptl3* ASO (50 mg/kg) halved the progression of *en face* atherosclerosis compared to the group receiving control ASO: 5.4% vs 11.4%. In these animals, administration of the GalNac modified ASO resulted in a 20 fold more potent suppression of ANGPTL3 with an ED$_{50}$ value equal to 10.4 mg (131).

Importantly, ANGPTL3-L$_{Rx}$ also reduces hepatic TG secretion, suggesting that a drug targeting ANGPTL3 would ameliorate hepatic steatosis, frequently associated to hypertriglyceridemia and insulin resistance. This effect was observed in diet-induced obese mice and ANGPTL3 deficient humans (131).

The monoclonal antibody anti-ANGPTL4 was developed by immunizing *Angptl4*$^{-/-}$ mice with the recombinant mouse protein for preclinical studies (132). The mAb 14D12 is directed to amino acids Gln29–His53 of the specific epitope 1 (SE1) (65). Hybridomas have been generated by the fusion of splenocytes which were isolated from an immunoresponsive mouse with NS1 myeloma cells. From this fusion, the hybridoma was identified to express IgGs that specifically inhibit LPL enzymatic activity. C57BL/6J mice

treated with mAb anti-ANGPTL4 had lower fasting TG when maintained on chow and HFD (-50% and -59%, respectively) compared to vehicle-treated mice (132). Interestingly, mAb anti-ANGPTL4 reduces TG also in *Ldlr*$^{-/-}$, ApoE$^{-/-}$ and db/db mice (132). Importantly, the inhibition of ANGPTL4 reduces instead total cholesterol in C57BL/6J mice. This effect was partially recapitulated in *Ldlr*$^{-/-}$ and db/db mice, but not in ApoE$^{-/-}$ mice (132). Interestingly, inhibition of ANGPTL4 showed a rapid drop in serum TG after an i.v. lipid challenge, indicating an increase of TG clearance. The inhibition of ANGPTL4 appears to decrease VLDL production, although this data was not confirmed by overexpressing ANGPTL4 with adenoviral (133) or transgene in adipose tissue and skeletal muscle (114).

Finally, gene editing through the CRISPR-Cas9 technology represents a new approach able to induce an ANGPLT3 permanent LOF mutation. A proof-of-concept study reported that injection of base editor 3 *Angptl3* into 5-week-old male mice resulted in a 49%, 31%, and 19% fall in the levels of ANGPTL3, TG and total cholesterol, respectively. When editing was carried on hyperlipidemic *Ldlr*$^{-/-}$ mice the reduction in TG and total cholesterol were 56% and 51%, respectively (134). However, clinical application of the gene editing approach as a pharmacological tool for preventing CVD remains questionable due to potential off-target mutagenesis, unknown toxicity or immunogenicity (135).

**7. Clinical development of ANGPTL3 inhibitors**

In a phase 1, first-in-human, clinical trial, safety and efficacy of evinacumab were tested after s.c. or i.v. injections in subjects with raised TG (150≤ TG ≤450 mg/dL) and/or LDL-C levels (≥ 100 mg/dL). The ascending single-dose were fixed to 75 mg, 150 mg or 250 mg for s.c. administration and to 5 mg/kg, 10 mg/kg or 20 mg/kg for the i.v. ones. Evinacumab was well tolerated and the most frequent emergent adverse events were headache (11.3%) and increase in ALT/AST2 enzymes, *i.e.* 2 treated subjects experiencing with >3X ULN. Compared to placebo, evinacumab reduced TG in a range between 1% (the lowest dose) to 75% (the highest dose) and LDL-C between 3.4% to 25.5% (136) (Table 2).

The results of two additional phase 1 trials with evinacumab have been recently reported (137). Subjects with TG levels between 150 and 450 mg/dL were randomized to two different treatment protocol, a single ascending dose study and a multiple ascending dose study. In the single ascending dose study, TG reduction was dose-dependent and rapid, with maximum drops at day 3. Dose-dependent reductions in TG were observed in both studies, with maximum reductions of 76.9% at day 3 with 10 mg/kg i.v. in the single ascending dose and of 83.1% at day 2 with 20 mg/kg *i.v.* Q4W in the multiple ascending dose study. Significant reductions were also observed in non-HDL-C, apoB, total cholesterol, HDL-C and apoA-I levels in most evinacumab treatment groups compared to placebo (137). Interestingly, evinacumab treatment in both studies did not result in significant changes in Lp(a) levels (137) (Table 2).

Evinacumab was also tested in nine adults with homozygous familial hypercholesterolemia for LDLR, including two null homozygotes and one compound heterozygote with two null alleles. Patients, already

taking aggressive lipid-lowering therapy, received evinacumab 250 mg *s.c.* at baseline and 15 mg/kg *i.v.* at week 2. After 4 weeks of treatment, evinacumab decreased LDL-C by a mean of 49±23% (range, 25 to 90), with an absolute decrease from baseline of 157±90 mg per deciliter (range, 71 to 323) (39). An approximately significant 48% reduction of apoB, non HDL-C and TG was also observed (39) (Table 2). Treatment was well tolerated, all nine patients reporting the occurrence of at least one adverse event, but no event led to treatment discontinuation. Thus, evinacumab was shown to efficiently reduce LDL-C and TG in homozygous patients already under intensive lipid lowering therapies. This evidence is in line with the fact that the lipid lowering effect of ANGPTL3 inhibitors are obtained in a LDLR-independent manner.

A phase 1 trial, in healthy volunteers aged 18 to 65 years, tested the pharmacokinetics, safety, tolerability and pharmacodynamics of single and multiple ascending doses of ANGPTL3-$L_{Rx}$ (131). Pharmacokinetic analysis of ANGPTL3-$L_{Rx}$ shows a linear and dose-dependent increase of maximum plasma concentrations ($C_{max}$) within 10 and 60 mg doses, after a rapid distribution phase. As the ANGPTL3-$L_{Rx}$ concentrations decreases, ANGPTL3 protein concentrations return toward baseline values. The calculated half-life ($t_{1/2}$) was approximately 3-5 weeks (131). ANGPTL3-$L_{RX}$ administered in a multiple-dose design was effective at day 43 to lower TG (from -33.2% to -63.1%), LDL-C (from -1.3% to -32.9%), VLDL-C (from -27.9% to -60%), non-HDL-C (from -10% to -36.6%), apoB (from -3.4% to -25.7%) and apoC-III (from -18.9% to -58.8%) compared to placebo group. At day 43, ANGPTL3 levels were reduced from baseline by 46.6% (10 mg), 72.5% (20 mg), 81.3% (40 mg) and 84.5% (60 mg). No clinical signs of prothrombotic effects, bleeding episodes, significant decreases in platelet counts and of liver or renal function damages were found (131) (Table2).

**8. Conclusions**

The magnitude of contribution of TG to CVD risk is evident both from long-term prospective studies (138) and genetic analyses (16). TGRL may penetrate the arterial wall by interacting with the positive charged residues on apoB and the negative charged groups on the arterial wall proteoglycans. This process allows TGRL to be retained within the sub-endothelial space and to undergo an oxidative modification which favors the development of atherosclerotic plaques and ASCVD (139). The lipolysis of TGRL rich in cholesterol and apoE releases oxidized FFA and lysolecithin which induce endothelial cell inflammation and coagulation (140). Recently, we have listed ANGPTL3 as an early predictor of peripheral artery disease, influencing the endothelial cell adhesion and stimulating the proliferation of haematopoietic stem cells, both processes exacerbating atherosclerosis (141).

Among pharmacological targets envisioned to reduce TG levels, activators of PPARs (*i.e.* fibrates being mild PPARα agonists) (10) have shown possible benefit in patients with primary hypertriglyceridemia, mixed hyperlipidemia and type 2 diabetes with raised TG and low HDL-C (6, 142-144). The efficacy of controlling the hypertriglyceridemia was also recently confirmed by the use of icosapent ethyl in patients with elevated TG levels despite the use of statins (145). The REDUCE-IT (Reduction of Cardiovascular Events with Icosapent

Ethyl–Intervention) trial demonstrated that after a median follow-up of 4.9 years, the primary endpoint, *i.e.* a composite of CV death, non-fatal MI, non-fatal stroke, coronary revascularization, or unstable angina, was reduced by 25% in the icosapent ethyl group *vs* placebo. Furthermore, icosapent ethyl was also superior to placebo to reduce total events, namely the occurrence of first and all recurrent major CV events by 30%. Specifically, first events fell by 25%, second ones by 32%, third ones by 31% and fourth ones or more by 48% (146). Of note, lomitapide, a first-in-class microsomal triglyceride transfer protein (MTP) inhibitor (147), has been shown to significantly control TG levels; it prevented pancreatitis in a patient with an inactivating mutation on the LPL, although with a potential long-term cost of hepatotoxicity (148). Despite these pharmacological opportunities, very effective and safe drugs for reducing TG levels are still missing. Within this scenario and along with the inhibition of apoC-III protein (149), the evidence of the role of ANGPTL3 and ANGPTL4 for controlling LPL activity and TG levels indicates a promising pharmacological target, although the adverse phenotype observed in Angptl4$^{-/-}$ mice (107, 112, 113), highlights ANGPTL3 as a better target. Although we are still at phase 1 of clinical development, the use of monoclonal antibodies and/or ASO directed to ANGPTL3 have shown very effective in lowering TG and LDL-C and increasing HDL-C (Figure 2). The ASO was safe, an important aspect, considering the thrombocytopenia found in patients given volanesorsen, an ASO against apoC-III , reducing chylomicron TG by roughly 83% (150). Thus, these therapies can be considered as a future valid implementation of the current use of PPAR-α agonists in the management of this very frequent clinical condition, namely hypertriglyceridemia.

**References**


1. W.E. Boden, D.L. Bhatt, P.P. Toth, K.K. Ray, M.J. Chapman, and T.F. Luscher. Profound reductions in first and total cardiovascular events with icosapent ethyl in the REDUCE-IT trial: why these results usher in a new era in dyslipidaemia therapeutics. Eur Heart J(2019).
2. S. Tsimikas. A Test in Context: Lipoprotein(a): Diagnosis, Prognosis, Controversies, and Emerging Therapies. J Am Coll Cardiol. 69:692-711 (2017).
3. M.B. Elshazly, P. Mani, S. Nissen, D.M. Brennan, D. Clark, S. Martin, S.R. Jones, R. Quispe, E. Donnellan, S.J. Nicholls, and R. Puri. Remnant cholesterol, coronary atheroma progression and clinical events in statin-treated patients with coronary artery disease. European journal of preventive cardiology:2047487319887578 (2019).
4. P.B. Sandesara, S.S. Virani, S. Fazio, and M.D. Shapiro. The Forgotten Lipids: Triglycerides, Remnant Cholesterol, and Atherosclerotic Cardiovascular Disease Risk. Endocrine reviews. 40:537-557 (2019).
5. N. Ferri, A. Corsini, C.R. Sirtori, and M. Ruscica. Present therapeutic role of cholesteryl ester transfer protein inhibitors. Pharmacological research. 128:29-41 (2018).
6. C.R. Sirtori, S. Yamashita, M. Francesca Greco, A. Corsini, F.W. G, and M. Ruscica. Recent advances in synthetic pharmacotherapies for dyslipidaemias. European journal of preventive cardiology:2047487319845314 (2019).
7. M.J. Chapman, H.N. Ginsberg, P. Amarenco, F. Andreotti, J. Boren, A.L. Catapano, O.S. Descamps, E. Fisher, P.T. Kovanen, J.A. Kuivenhoven, P. Lesnik, L. Masana, B.G. Nordestgaard, K.K. Ray, Z. Reiner, M.R. Taskinen, L. Tokgozoglu, A. Tybjaerg-Hansen, G.F. Watts, and P. European Atherosclerosis Society Consensus. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. Eur Heart J. 32:1345-1361 (2011).
8. F. Mach, C. Baigent, A.L. Catapano, K.C. Koskinas, M. Casula, L. Badimon, M.J. Chapman, G.G. De Backer, V. Delgado, B.A. Ference, I.M. Graham, A. Halliday, U. Landmesser, B. Mihaylova, T.R. Pedersen, G. Riccardi, D.J. Richter, M.S. Sabatine, M.R. Taskinen, L. Tokgozoglu, O. Wiklund, and E.S.C.S.D. Group. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 41:111-188 (2020).
9. C.J. Packard. Triglyceride lowering 2.0: back to the future? Eur Heart J. 41:95-98 (2020).
10. J.C. Fruchart, R.D. Santos, C. Aguilar-Salinas, M. Aikawa, K. Al Rasadi, P. Amarenco, P.J. Barter, R. Ceska, A. Corsini, J.P. Despres, P. Duriez, R.H. Eckel, M.V. Ezhov, M. Farnier, H.N. Ginsberg, M.P. Hermans, S. Ishibashi, F. Karpe, T. Kodama, W. Koenig, M. Krempf, S. Lim, A.J. Lorenzatti, R. McPherson, J.M. Nunez-Cortes, B.G. Nordestgaard, H. Ogawa, C.J. Packard, J. Plutzky, C.I. Ponte-Negretti, A. Pradhan, K.K. Ray, Z. Reiner, P.M. Ridker, M. Ruscica, S. Sadikot, H. Shimano, P. Sritara, J.K. Stock, T.C. Su, A.V. Susekov, A. Tartar, M.R. Taskinen, A. Tenenbaum, L.S. Tokgozoglu, B.

Tomlinson, A. Tybjaerg-Hansen, P. Valensi, M. Vrablik, W. Wahli, G.F. Watts, S. Yamashita, K. Yokote, A. Zambon, and P. Libby. The selective peroxisome proliferator-activated receptor alpha modulator (SPPARMalpha) paradigm: conceptual framework and therapeutic potential : A consensus statement from the International Atherosclerosis Society (IAS) and the Residual Risk Reduction Initiative (R3i) Foundation. Cardiovascular diabetology. 18:71 (2019).

11. A.D. Sniderman, G. Thanassoulis, T. Glavinovic, A.M. Navar, M. Pencina, A. Catapano, and B.A. Ference. Apolipoprotein B Particles and Cardiovascular Disease: A Narrative Review. JAMA cardiology(2019).

12. A.D. Sniderman, P. Couture, S.S. Martin, J. DeGraaf, P.R. Lawler, W.C. Cromwell, J.T. Wilkins, and G. Thanassoulis. Hypertriglyceridemia and cardiovascular risk: a cautionary note about metabolic confounding. J Lipid Res. 59:1266-1275 (2018).

13. N.A. Marston, R.P. Giugliano, K. Im, M.G. Silverman, M.L. O'Donoghue, S.D. Wiviott, B.A. Ference, and M.S. Sabatine. Association Between Triglyceride Lowering and Reduction of Cardiovascular Risk Across Multiple Lipid-Lowering Therapeutic Classes: A Systematic Review and Meta-Regression Analysis of Randomized Controlled Trials. Circulation. 140:1308-1317 (2019).

14. R. Do, C.J. Willer, E.M. Schmidt, S. Sengupta, C. Gao, G.M. Peloso, S. Gustafsson, S. Kanoni, A. Ganna, J. Chen, M.L. Buchkovich, S. Mora, J.S. Beckmann, J.L. Bragg-Gresham, H.Y. Chang, A. Demirkan, H.M. Den Hertog, L.A. Donnelly, G.B. Ehret, T. Esko, M.F. Feitosa, T. Ferreira, K. Fischer, P. Fontanillas, R.M. Fraser, D.F. Freitag, D. Gurdasani, K. Heikkila, E. Hypponen, A. Isaacs, A.U. Jackson, A. Johansson, T. Johnson, M. Kaakinen, J. Kettunen, M.E. Kleber, X. Li, J. Luan, L.P. Lyytikainen, P.K. Magnusson, M. Mangino, E. Mihailov, M.E. Montasser, M. Muller-Nurasyid, I.M. Nolte, J.R. O'Connell, C.D. Palmer, M. Perola, A.K. Petersen, S. Sanna, R. Saxena, S.K. Service, S. Shah, D. Shungin, C. Sidore, C. Song, R.J. Strawbridge, I. Surakka, T. Tanaka, T.M. Teslovich, G. Thorleifsson, E.G. Van den Herik, B.F. Voight, K.A. Volcik, L.L. Waite, A. Wong, Y. Wu, W. Zhang, D. Absher, G. Asiki, I. Barroso, L.F. Been, J.L. Bolton, L.L. Bonnycastle, P. Brambilla, M.S. Burnett, G. Cesana, M. Dimitriou, A.S. Doney, A. Doring, P. Elliott, S.E. Epstein, G.I. Eyjolfsson, B. Gigante, M.O. Goodarzi, H. Grallert, M.L. Gravito, C.J. Groves, G. Hallmans, A.L. Hartikainen, C. Hayward, D. Hernandez, A.A. Hicks, H. Holm, Y.J. Hung, T. Illig, M.R. Jones, P. Kaleebu, J.J. Kastelein, K.T. Khaw, E. Kim, N. Klopp, P. Komulainen, M. Kumari, C. Langenberg, T. Lehtimaki, S.Y. Lin, J. Lindstrom, R.J. Loos, F. Mach, W.L. McArdle, C. Meisinger, B.D. Mitchell, G. Muller, R. Nagaraja, N. Narisu, T.V. Nieminen, R.N. Nsubuga, I. Olafsson, K.K. Ong, A. Palotie, T. Papamarkou, C. Pomilla, A. Pouta, D.J. Rader, M.P. Reilly, P.M. Ridker, F. Rivadeneira, I. Rudan, A. Ruokonen, N. Samani, H. Scharnagl, J. Seeley, K. Silander, A. Stancakova, K. Stirrups, A.J. Swift, L. Tiret, A.G. Uitterlinden, L.J. van Pelt, S. Vedantam, N. Wainwright, C. Wijmenga, S.H. Wild, G. Willemsen, T. Wilsgaard, J.F. Wilson, E.H. Young, J.H. Zhao, L.S. Adair, D. Arveiler, T.L. Assimes, S. Bandinelli, F. Bennett, M. Bochud, B.O. Boehm, D.I. Boomsma, I.B. Borecki, S.R. Bornstein, P. Bovet,

M. Burnier, H. Campbell, A. Chakravarti, J.C. Chambers, Y.D. Chen, F.S. Collins, R.S. Cooper, J. Danesh, G. Dedoussis, U. de Faire, A.B. Feranil, J. Ferrieres, L. Ferrucci, N.B. Freimer, C. Gieger, L.C. Groop, V. Gudnason, U. Gyllensten, A. Hamsten, T.B. Harris, A. Hingorani, J.N. Hirschhorn, A. Hofman, G.K. Hovingh, C.A. Hsiung, S.E. Humphries, S.C. Hunt, K. Hveem, C. Iribarren, M.R. Jarvelin, A. Jula, M. Kahonen, J. Kaprio, A. Kesaniemi, M. Kivimaki, J.S. Kooner, P.J. Koudstaal, R.M. Krauss, D. Kuh, J. Kuusisto, K.O. Kyvik, M. Laakso, T.A. Lakka, L. Lind, C.M. Lindgren, N.G. Martin, W. Marz, M.I. McCarthy, C.A. McKenzie, P. Meneton, A. Metspalu, L. Moilanen, A.D. Morris, P.B. Munroe, I. Njolstad, N.L. Pedersen, C. Power, P.P. Pramstaller, J.F. Price, B.M. Psaty, T. Quertermous, R. Rauramaa, D. Saleheen, V. Salomaa, D.K. Sanghera, J. Saramies, P.E. Schwarz, W.H. Sheu, A.R. Shuldiner, A. Siegbahn, T.D. Spector, K. Stefansson, D.P. Strachan, B.O. Tayo, E. Tremoli, J. Tuomilehto, M. Uusitupa, C.M. van Duijn, P. Vollenweider, L. Wallentin, N.J. Wareham, J.B. Whitfield, B.H. Wolffenbuttel, D. Altshuler, J.M. Ordovas, E. Boerwinkle, C.N. Palmer, U. Thorsteinsdottir, D.I. Chasman, J.I. Rotter, P.W. Franks, S. Ripatti, L.A. Cupples, M.S. Sandhu, S.S. Rich, M. Boehnke, P. Deloukas, K.L. Mohlke, E. Ingelsson, G.R. Abecasis, M.J. Daly, B.M. Nealeand S. Kathiresan. Common variants associated with plasma triglycerides and risk for coronary artery disease. Nat Genet. 45:1345-1352 (2013).

15. A.B. Jorgensen, R. Frikke-Schmidt, A.S. West, P. Grande, B.G. Nordestgaard, and A. Tybjaerg-Hansen. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. Eur Heart J. 34:1826-1833 (2013).

16. B.A. Ference, J.J.P. Kastelein, K.K. Ray, H.N. Ginsberg, M.J. Chapman, C.J. Packard, U. Laufs, C. Oliver-Williams, A.M. Wood, A.S. Butterworth, E. Di Angelantonio, J. Danesh, S.J. Nicholls, D.L. Bhatt, M.S. Sabatine, and A.L. Catapano. Association of Triglyceride-Lowering LPL Variants and LDL-C-Lowering LDLR Variants With Risk of Coronary Heart Disease. JAMA. 321:364-373 (2019).

17. J.R. Mead, S.A. Irvine, and D.P. Ramji. Lipoprotein lipase: structure, function, regulation, and role in disease. Journal of molecular medicine. 80:753-769 (2002).

18. B.S. Davies, A.P. Beigneux, R.H. Barnes, 2nd, Y. Tu, P. Gin, M.M. Weinstein, C. Nobumori, R. Nyren, I. Goldberg, G. Olivecrona, A. Bensadoun, S.G. Young, and L.G. Fong. GPIHBP1 is responsible for the entry of lipoprotein lipase into capillaries. Cell metabolism. 12:42-52 (2010).

19. S.G. Youngand R. Zechner. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. Genes & development. 27:459-484 (2013).

20. R.X. Ioka, M.J. Kang, S. Kamiyama, D.H. Kim, K. Magoori, A. Kamataki, Y. Ito, Y.A. Takei, M. Sasaki, T. Suzuki, H. Sasano, S. Takahashi, J. Sakai, T. Fujino, and T.T. Yamamoto. Expression cloning and characterization of a novel glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein, GPI-HBP1. J Biol Chem. 278:7344-7349 (2003).

21. A.P. Beigneux, B.S. Davies, P. Gin, M.M. Weinstein, E. Farber, X. Qiao, F. Peale, S. Bunting, R.L. Walzem, J.S. Wong, W.S. Blaner, Z.M. Ding, K. Melford, N. Wongsiriroj, X. Shu, F. de Sauvage, R.O. Ryan, L.G. Fong, A. Bensadoun, and S.G. Young. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. Cell metabolism. 5:279-291 (2007).

22. S.G. Young, B.S. Davies, L.G. Fong, P. Gin, M.M. Weinstein, A. Bensadoun, and A.P. Beigneux. GPIHBP1: an endothelial cell molecule important for the lipolytic processing of chylomicrons. Curr Opin Lipidol. 18:389-396 (2007).

23. A.P. Beigneux, B.S. Davies, A. Bensadoun, L.G. Fong, and S.G. Young. GPIHBP1, a GPI-anchored protein required for the lipolytic processing of triglyceride-rich lipoproteins. J Lipid Res. 50 Suppl:S57-62 (2009).

24. M. Botta, E. Maurer, M. Ruscica, S. Romeo, T.M. Stulnig, and P. Pingitore. Deciphering the role of V200A and N291S mutations leading to LPL deficiency. Atherosclerosis. 282:45-51 (2019).

25. C.M. Allan, M. Larsson, R.S. Jung, M. Ploug, A. Bensadoun, A.P. Beigneux, L.G. Fong, and S.G. Young. Mobility of "HSPG-bound" LPL explains how LPL is able to reach GPIHBP1 on capillaries. J Lipid Res. 58:216-225 (2017).

26. R. Arora, A.V. Nimonkar, D. Baird, C. Wang, C.H. Chiu, P.A. Horton, S. Hanrahan, R. Cubbon, S. Weldon, W.R. Tschantz, S. Mueller, R. Brunner, P. Lehr, P. Meier, J. Ottl, A. Voznesensky, P. Pandey, T.M. Smith, A. Stojanovic, A. Flyer, T.E. Benson, M.J. Romanowski, and J.W. Trauger. Structure of lipoprotein lipase in complex with GPIHBP1. Proc Natl Acad Sci U S A. 116:10360-10365 (2019).

27. A.V. Nimonkar, S. Weldon, K. Godbout, D. Panza, S. Hanrahan, R. Cubbon, F. Xu, J.W. Trauger, J. Gao, and A. Voznesensky. A lipoprotein lipase --GPI-anchored high density lipoprotein binding protein 1 fusion lowers triglycerides in mice: implications for managing familial chylomicronemia syndrome. J Biol Chem(2019).

28. W.K. Sonnenburg, D. Yu, E.C. Lee, W. Xiong, G. Gololobov, B. Key, J. Gay, N. Wilganowski, Y. Hu, S. Zhao, M. Schneider, Z.M. Ding, B.P. Zambrowicz, G. Landes, D.R. Powell, and U. Desai. GPIHBP1 stabilizes lipoprotein lipase and prevents its inhibition by angiopoietin-like 3 and angiopoietin-like 4. J Lipid Res. 50:2421-2429 (2009).

29. S. Mysling, K.K. Kristensen, M. Larsson, A.P. Beigneux, H. Gardsvoll, L.G. Fong, A. Bensadouen, T.J. Jorgensen, S.G. Young, and M. Ploug. The acidic domain of the endothelial membrane protein GPIHBP1 stabilizes lipoprotein lipase activity by preventing unfolding of its catalytic domain. eLife. 5:e12095 (2016).

30. S.N. Vallerieand K.E. Bornfeldt. GPIHBP1: two get tangled. Circ Res. 116:560-562 (2015).

31. A. Baass, M. Paquette, S. Bernard, and R.A. Hegele. Familial Chylomicronemia Syndrome: an under-recognized cause of severe hypertriglyceridemia. Journal of internal medicine(2019).

32. A.P. Beigneux, K. Miyashita, M. Ploug, D.J. Blom, M. Ai, M.F. Linton, W. Khovidhunkit, R. Dufour, A. Garg, M.A. McMahon, C.R. Pullinger, N.P. Sandoval, X. Hu, C.M. Allan, M. Larsson, T. Machida, M. Murakami, K. Reue, P. Tontonoz, I.J. Goldberg, P. Moulin, S. Charriere, L.G. Fong, K. Nakajima, and S.G. Young. Autoantibodies against GPIHBP1 as a Cause of Hypertriglyceridemia. N Engl J Med. 376:1647-1658 (2017).

33. B.G. Nordestgaard, S. Abildgaard, H.H. Wittrup, R. Steffensen, G. Jensen, and A. Tybjaerg-Hansen. Heterozygous lipoprotein lipase deficiency: frequency in the general population, effect on plasma lipid levels, and risk of ischemic heart disease. Circulation. 96:1737-1744 (1997).

34. G. Myocardial Infarction, C.A.E.C. Investigators, N.O. Stitziel, K.E. Stirrups, N.G. Masca, J. Erdmann, P.G. Ferrario, I.R. Konig, P.E. Weeke, T.R. Webb, P.L. Auer, U.M. Schick, Y. Lu, H. Zhang, M.P. Dube, A. Goel, M. Farrall, G.M. Peloso, H.H. Won, R. Do, E. van Iperen, S. Kanoni, J. Kruppa, A. Mahajan, R.A. Scott, C. Willenberg, P.S. Braund, J.C. van Capelleveen, A.S. Doney, L.A. Donnelly, R. Asselta, P.A. Merlini, S. Duga, N. Marziliano, J.C. Denny, C.M. Shaffer, N.E. El-Mokhtari, A. Franke, O. Gottesman, S. Heilmann, C. Hengstenberg, P. Hoffman, O.L. Holmen, K. Hveem, J.H. Jansson, K.H. Jockel, T. Kessler, J. Kriebel, K.L. Laugwitz, E. Marouli, N. Martinelli, M.I. McCarthy, N.R. Van Zuydam, C. Meisinger, T. Esko, E. Mihailov, S.A. Escher, M. Alver, S. Moebus, A.D. Morris, M. Muller-Nurasyid, M. Nikpay, O. Olivieri, L.P. Lemieux Perreault, A. AlQarawi, N.R. Robertson, K.O. Akinsanya, D.F. Reilly, T.F. Vogt, W. Yin, F.W. Asselbergs, C. Kooperberg, R.D. Jackson, E. Stahl, K. Strauch, T.V. Varga, M. Waldenberger, L. Zeng, A.T. Kraja, C. Liu, G.B. Ehret, C. Newton-Cheh, D.I. Chasman, R. Chowdhury, M. Ferrario, I. Ford, J.W. Jukema, F. Kee, K. Kuulasmaa, B.G. Nordestgaard, M. Perola, D. Saleheen, N. Sattar, P. Surendran, D. Tregouet, R. Young, J.M. Howson, A.S. Butterworth, J. Danesh, D. Ardissino, E.P. Bottinger, R. Erbel, P.W. Franks, D. Girelli, A.S. Hall, G.K. Hovingh, A. Kastrati, W. Lieb, T. Meitinger, W.E. Kraus, S.H. Shah, R. McPherson, M. Orho-Melander, O. Melander, A. Metspalu, C.N. Palmer, A. Peters, D. Rader, M.P. Reilly, R.J. Loos, A.P. Reiner, D.M. Roden, J.C. Tardif, J.R. Thompson, N.J. Wareham, H. Watkins, C.J. Willer, S. Kathiresan, P. Deloukas, N.J. Samaniand H. Schunkert. Coding Variation in ANGPTL4, LPL, and SVEP1 and the Risk of Coronary Disease. N Engl J Med. 374:1134-1144 (2016).

35. A.B. Jorgensen, R. Frikke-Schmidt, B.G. Nordestgaard, and A. Tybjaerg-Hansen. Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med. 371:32-41 (2014).

36. A.V. Khera, H.H. Won, G.M. Peloso, C. O'Dushlaine, D. Liu, N.O. Stitziel, P. Natarajan, A. Nomura, C.A. Emdin, N. Gupta, I.B. Borecki, R. Asselta, S. Duga, P.A. Merlini, A. Correa, T. Kessler, J.G. Wilson, M.J. Bown, A.S. Hall, P.S. Braund, D.J. Carey, M.F. Murray, H.L. Kirchner, J.B. Leader, D.R. Lavage, J.N. Manus, D.N. Hartzel, N.J. Samani, H. Schunkert, J. Marrugat, R. Elosua, R. McPherson, M. Farrall, H. Watkins, E.S. Lander, D.J. Rader, J. Danesh, D. Ardissino, S. Gabriel, C. Willer, G.R. Abecasis, D. Saleheen, F.E. Dewey, S. Kathiresan, D.S.G.C.E.C. Myocardial Infarction Genetics Consortium, and C.

Global Lipids Genetics. Association of Rare and Common Variation in the Lipoprotein Lipase Gene With Coronary Artery Disease. JAMA. 317:937-946 (2017).

37. F.E. Dewey, V. Gusarova, C. O'Dushlaine, O. Gottesman, J. Trejos, C. Hunt, C.V. Van Hout, L. Habegger, D. Buckler, K.M. Lai, J.B. Leader, M.F. Murray, M.D. Ritchie, H.L. Kirchner, D.H. Ledbetter, J. Penn, A. Lopez, I.B. Borecki, J.D. Overton, J.G. Reid, D.J. Carey, A.J. Murphy, G.D. Yancopoulos, A. Baras, J. Gromada, and A.R. Shuldiner. Inactivating Variants in ANGPTL4 and Risk of Coronary Artery Disease. N Engl J Med. 374:1123-1133 (2016).

38. F.E. Dewey, V. Gusarova, R.L. Dunbar, C. O'Dushlaine, C. Schurmann, O. Gottesman, S. McCarthy, C.V. Van Hout, S. Bruse, H.M. Dansky, J.B. Leader, M.F. Murray, M.D. Ritchie, H.L. Kirchner, L. Habegger, A. Lopez, J. Penn, A. Zhao, W. Shao, N. Stahl, A.J. Murphy, S. Hamon, A. Bouzelmat, R. Zhang, B. Shumel, R. Pordy, D. Gipe, G.A. Herman, W.H.H. Sheu, I.T. Lee, K.W. Liang, X. Guo, J.I. Rotter, Y.I. Chen, W.E. Kraus, S.H. Shah, S. Damrauer, A. Small, D.J. Rader, A.B. Wulff, B.G. Nordestgaard, A. Tybjaerg-Hansen, A.M. van den Hoek, H.M.G. Princen, D.H. Ledbetter, D.J. Carey, J.D. Overton, J.G. Reid, W.J. Sasiela, P. Banerjee, A.R. Shuldiner, I.B. Borecki, T.M. Teslovich, G.D. Yancopoulos, S.J. Mellis, J. Gromada, and A. Baras. Genetic and Pharmacologic Inactivation of ANGPTL3 and Cardiovascular Disease. N Engl J Med. 377:211-221 (2017).

39. D. Gaudet, D.A. Gipe, R. Pordy, Z. Ahmad, M. Cuchel, P.K. Shah, K.Y. Chyu, W.J. Sasiela, K.C. Chan, D. Brisson, E. Khoury, P. Banerjee, V. Gusarova, J. Gromada, N. Stahl, G.D. Yancopoulos, and G.K. Hovingh. ANGPTL3 Inhibition in Homozygous Familial Hypercholesterolemia. N Engl J Med. 377:296-297 (2017).

40. D. Gaudet, V.J. Alexander, B.F. Baker, D. Brisson, K. Tremblay, W. Singleton, R.S. Geary, S.G. Hughes, N.J. Viney, M.J. Graham, R.M. Crooke, J.L. Witztum, J.D. Brunzell, and J.J. Kastelein. Antisense Inhibition of Apolipoprotein C-III in Patients with Hypertriglyceridemia. N Engl J Med. 373:438-447 (2015).

41. M. Alves-Bezerraand D.E. Cohen. Triglyceride Metabolism in the Liver. Comprehensive Physiology. 8:1-8 (2017).

42. S.Y. Kim, S.M. Park, and S.T. Lee. Apolipoprotein C-II is a novel substrate for matrix metalloproteinases. Biochem Biophys Res Commun. 339:47-54 (2006).

43. S. Romeo, W. Yin, J. Kozlitina, L.A. Pennacchio, E. Boerwinkle, H.H. Hobbs, and J.C. Cohen. Rare loss-of-function mutations in ANGPTL family members contribute to plasma triglyceride levels in humans. J Clin Invest. 119:70-79 (2009).

44. A. Koster, Y.B. Chao, M. Mosior, A. Ford, P.A. Gonzalez-DeWhitt, J.E. Hale, D. Li, Y. Qiu, C.C. Fraser, D.D. Yang, J.G. Heuer, S.R. Jaskunas, and P. Eacho. Transgenic angiopoietin-like (angptl)4 overexpression and targeted disruption of angptl4 and angptl3: regulation of triglyceride metabolism. Endocrinology. 146:4943-4950 (2005).

45. F. Quagliarini, Y. Wang, J. Kozlitina, N.V. Grishin, R. Hyde, E. Boerwinkle, D.M. Valenzuela, A.J. Murphy, J.C. Cohen, and H.H. Hobbs. Atypical angiopoietin-like protein that regulates ANGPTL3. Proc Natl Acad Sci U S A. 109:19751-19756 (2012).
46. M.C. Jong, M.H. Hofker, and L.M. Havekes. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. Arterioscler Thromb Vasc Biol. 19:472-484 (1999).
47. S. Davis, T.H. Aldrich, P.F. Jones, A. Acheson, D.L. Compton, V. Jain, T.E. Ryan, J. Bruno, C. Radziejewski, P.C. Maisonpierre, and G.D. Yancopoulos. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell. 87:1161-1169 (1996).
48. P.C. Maisonpierre, C. Suri, P.F. Jones, S. Bartunkova, S.J. Wiegand, C. Radziejewski, D. Compton, J. McClain, T.H. Aldrich, N. Papadopoulos, T.J. Daly, S. Davis, T.N. Sato, and G.D. Yancopoulos. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science. 277:55-60 (1997).
49. M.G. Lupoand N. Ferri. Angiopoietin-Like 3 (ANGPTL3) and Atherosclerosis: Lipid and Non-Lipid Related Effects. Journal of cardiovascular development and disease. 5:(2018).
50. D. Conklin, D. Gilbertson, D.W. Taft, M.F. Maurer, T.E. Whitmore, D.L. Smith, K.M. Walker, L.H. Chen, S. Wattler, M. Nehls, and K.B. Lewis. Identification of a mammalian angiopoietin-related protein expressed specifically in liver. Genomics. 62:477-482 (1999).
51. Y. Li, L. Sun, H. Xu, Z. Fang, W. Yao, W. Guo, J. Rao, and X. Zha. Angiopoietin-like protein 3 modulates barrier properties of human glomerular endothelial cells through a possible signaling pathway involving phosphatidylinositol-3 kinase/protein kinase B and integrin alphaVbeta3. Acta biochimica et biophysica Sinica. 40:459-465 (2008).
52. Y. Lin, J. Rao, X.L. Zha, and H. Xu. Angiopoietin-like 3 induces podocyte F-actin rearrangement through integrin alpha(V)beta(3)/FAK/PI3K pathway-mediated Rac1 activation. BioMed research international. 2013:135608 (2013).
53. R. Dai, Y. Lin, H. Liu, J. Rao, Y. Zhai, X. Zha, X. Fang, and H. Xu. A vital role for Angptl3 in the PAN-induced podocyte loss by affecting detachment and apoptosis in vitro. BMC nephrology. 16:38 (2015).
54. M. Ono, T. Shimizugawa, M. Shimamura, K. Yoshida, C. Noji-Sakikawa, Y. Ando, R. Koishi, and H. Furukawa. Protein region important for regulation of lipid metabolism in angiopoietin-like 3 (ANGPTL3): ANGPTL3 is cleaved and activated in vivo. J Biol Chem. 278:41804-41809 (2003).
55. G. Camenisch, M.T. Pisabarro, D. Sherman, J. Kowalski, M. Nagel, P. Hass, M.H. Xie, A. Gurney, S. Bodary, X.H. Liang, K. Clark, M. Beresini, N. Ferrara, and H.P. Gerber. ANGPTL3 stimulates endothelial cell adhesion and migration via integrin alpha vbeta 3 and induces blood vessel formation in vivo. J Biol Chem. 277:17281-17290 (2002).

56. R. Essalmani, D. Susan-Resiga, A. Chamberland, M.C. Asselin, M. Canuel, D. Constam, J.W. Creemers, R. Day, D. Gauthier, A. Prat, and N.G. Seidah. Furin is the primary in vivo convertase of angiopoietin-like 3 and endothelial lipase in hepatocytes. J Biol Chem. 288:26410-26418 (2013).

57. P. Dongiovanni, M. Meroni, G. Baselli, R.M. Mancina, M. Ruscica, M. Longo, R. Rametta, A. Cespiati, S. Pelusi, N. Ferri, V. Ranzani, V. Nobili, J. Pihlajamaki, A.L. Fracanzani, S. Badiali, S. Petta, S. Fargion, S. Romeo, J. Kozlitina, and L. Valenti. PCSK7 gene variation bridges atherogenic dyslipidemia with hepatic inflammation in NAFLD patients. J Lipid Res. 60:1144-1153 (2019).

58. P. Zhu, Y.Y. Goh, H.F. Chin, S. Kersten, and N.S. Tan. Angiopoietin-like 4: a decade of research. Bioscience reports. 32:211-219 (2012).

59. I. Kim, H.G. Kim, H. Kim, H.H. Kim, S.K. Park, C.S. Uhm, Z.H. Lee, and G.Y. Koh. Hepatic expression, synthesis and secretion of a novel fibrinogen/angiopoietin-related protein that prevents endothelial-cell apoptosis. Biochem J. 346 Pt 3:603-610 (2000).

60. S. Kersten, S. Mandard, N.S. Tan, P. Escher, D. Metzger, P. Chambon, F.J. Gonzalez, B. Desvergne, and W. Wahli. Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. J Biol Chem. 275:28488-28493 (2000).

61. J.C. Yoon, T.W. Chickering, E.D. Rosen, B. Dussault, Y. Qin, A. Soukas, J.M. Friedman, W.E. Holmes, and B.M. Spiegelman. Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. Molecular and cellular biology. 20:5343-5349 (2000).

62. W. Yin, S. Romeo, S. Chang, N.V. Grishin, H.H. Hobbs, and J.C. Cohen. Genetic variation in ANGPTL4 provides insights into protein processing and function. J Biol Chem. 284:13213-13222 (2009).

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

64. F. Mattijssenand S. Kersten. Regulation of triglyceride metabolism by Angiopoietin-like proteins. Biochimica et biophysica acta. 1821:782-789 (2012).

65. E.C. Lee, U. Desai, G. Gololobov, S. Hong, X. Feng, X.C. Yu, J. Gay, N. Wilganowski, C. Gao, L.L. Du, J. Chen, Y. Hu, S. Zhao, L. Kirkpatrick, M. Schneider, B.P. Zambrowicz, G. Landes, D.R. Powell, and W.K. Sonnenburg. Identification of a new functional domain in angiopoietin-like 3 (ANGPTL3) and angiopoietin-like 4 (ANGPTL4) involved in binding and inhibition of lipoprotein lipase (LPL). J Biol Chem. 284:13735-13745 (2009).

66. M.H. Yau, Y. Wang, K.S. Lam, J. Zhang, D. Wu, and A. Xu. A highly conserved motif within the NH2-terminal coiled-coil domain of angiopoietin-like protein 4 confers its inhibitory effects on lipoprotein lipase by disrupting the enzyme dimerization. J Biol Chem. 284:11942-11952 (2009).

67. A. Siddiqa, J. Ahmad, A. Ali, R.Z. Paracha, Z. Bibi, and B. Aslam. Structural characterization of ANGPTL8 (betatrophin) with its interacting partner lipoprotein lipase. Computational biology and chemistry. 61:210-220 (2016).

68. G. Ren, J.Y. Kim, and C.M. Smas. Identification of RIFL, a novel adipocyte-enriched insulin target gene with a role in lipid metabolism. American journal of physiology Endocrinology and metabolism. 303:E334-351 (2012).

69. R. Zhang. Lipasin, a novel nutritionally-regulated liver-enriched factor that regulates serum triglyceride levels. Biochemical and biophysical research communications. 424:786-792 (2012).

70. R. Kaplan, T. Zhang, M. Hernandez, F.X. Gan, S.D. Wright, M.G. Waters, and T.Q. Cai. Regulation of the angiopoietin-like protein 3 gene by LXR. J Lipid Res. 44:136-143 (2003).

71. T. Inaba, M. Matsuda, M. Shimamura, N. Takei, N. Terasaka, Y. Ando, H. Yasumo, R. Koishi, M. Makishima, and I. Shimomura. Angiopoietin-like protein 3 mediates hypertriglyceridemia induced by the liver X receptor. J Biol Chem. 278:21344-21351 (2003).

72. Y. Zhang, J.J. Repa, K. Gauthier, and D.J. Mangelsdorf. Regulation of lipoprotein lipase by the oxysterol receptors, LXRalpha and LXRbeta. The Journal of biological chemistry. 276:43018-43024 (2001).

73. J. Lee, S.W. Hong, S.E. Park, E.J. Rhee, C.Y. Park, K.W. Oh, S.W. Park, and W.Y. Lee. AMP-activated protein kinase suppresses the expression of LXR/SREBP-1 signaling-induced ANGPTL8 in HepG2 cells. Molecular and cellular endocrinology. 414:148-155 (2015).

74. C. Fugier, J.J. Tousaint, X. Prieur, M. Plateroti, J. Samarut, and P. Delerive. The lipoprotein lipase inhibitor ANGPTL3 is negatively regulated by thyroid hormone. J Biol Chem. 281:11553-11559 (2006).

75. K. Inukai, Y. Nakashima, M. Watanabe, S. Kurihara, T. Awata, H. Katagiri, Y. Oka, and S. Katayama. ANGPTL3 is increased in both insulin-deficient and -resistant diabetic states. Biochemical and biophysical research communications. 317:1075-1079 (2004).

76. M. Shimamura, M. Matsuda, Y. Ando, R. Koishi, H. Yasumo, H. Furukawa, and I. Shimomura. Leptin and insulin down-regulate angiopoietin-like protein 3, a plasma triglyceride-increasing factor. Biochem Biophys Res Commun. 322:1080-1085 (2004).

77. M. Botta, M. Audano, A. Sahebkar, C.R. Sirtori, N. Mitro, and M. Ruscica. PPAR Agonists and Metabolic Syndrome: An Established Role? International journal of molecular sciences. 19:(2018).

78. H. Ge, J.Y. Cha, H. Gopal, C. Harp, X. Yu, J.J. Repa, and C. Li. Differential regulation and properties of angiopoietin-like proteins 3 and 4. J Lipid Res. 46:1484-1490 (2005).

79. H. Staiger, C. Haas, J. Machann, R. Werner, M. Weisser, F. Schick, F. Machicao, N. Stefan, A. Fritsche, and H.U. Haring. Muscle-derived angiopoietin-like protein 4 is induced by fatty acids via peroxisome proliferator-activated receptor (PPAR)-delta and is of metabolic relevance in humans. Diabetes. 58:579-589 (2009).

80. X. Yu, S.C. Burgess, H. Ge, K.K. Wong, R.H. Nassem, D.J. Garry, A.D. Sherry, C.R. Malloy, J.P. Berger, and C. Li. Inhibition of cardiac lipoprotein utilization by transgenic overexpression of Angptl4 in the heart. Proc Natl Acad Sci U S A. 102:1767-1772 (2005).

81. L. Yang, R. Yin, Z. Wang, X. Wang, Y. Zhang, and D. Zhao. Circulating Angptl3 and Angptl8 Are Increased in Patients with Hypothyroidism. BioMed research international. 2019:3814687 (2019).

82. T. Shimizugawa, M. Ono, M. Shimamura, K. Yoshida, Y. Ando, R. Koishi, K. Ueda, T. Inaba, H. Minekura, T. Kohama, and H. Furukawa. ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. J Biol Chem. 277:33742-33748 (2002).

83. M. Shimamura, M. Matsuda, S. Kobayashi, Y. Ando, M. Ono, R. Koishi, H. Furukawa, M. Makishima, and I. Shimomura. Angiopoietin-like protein 3, a hepatic secretory factor, activates lipolysis in adipocytes. Biochem Biophys Res Commun. 301:604-609 (2003).

84. J.R. Meadand D.P. Ramji. The pivotal role of lipoprotein lipase in atherosclerosis. Cardiovascular research. 55:261-269 (2002).

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

86. M.G. McCoy, G.S. Sun, D. Marchadier, C. Maugeais, J.M. Glick, and D.J. Rader. Characterization of the lipolytic activity of endothelial lipase. Journal of lipid research. 43:921-929 (2002).

87. J. Liu, H. Afroza, D.J. Rader, and W. Jin. Angiopoietin-like protein 3 inhibits lipoprotein lipase activity through enhancing its cleavage by proprotein convertases. The Journal of biological chemistry. 285:27561-27570 (2010).

88. J.F. Haller, I.J. Mintah, L.M. Shihanian, P. Stevis, D. Buckler, C.A. Alexa-Braun, S. Kleiner, S. Banfi, J.C. Cohen, H.H. Hobbs, G.D. Yancopoulos, A.J. Murphy, V. Gusarova, and J. Gromada. ANGPTL8 requires ANGPTL3 to inhibit lipoprotein lipase and plasma triglyceride clearance. J Lipid Res. 58:1166-1173 (2017).

89. X. Chi, E.C. Britt, H.W. Shows, A.J. Hjelmaas, S.K. Shetty, E.M. Cushing, W. Li, A. Dou, R. Zhang, and B.S.J. Davies. ANGPTL8 promotes the ability of ANGPTL3 to bind and inhibit lipoprotein lipase. Molecular metabolism. 6:1137-1149 (2017).

90. O. Kovrov, K.K. Kristensen, E. Larsson, M. Ploug, and G. Olivecrona. On the mechanism of angiopoietin-like protein 8 for control of lipoprotein lipase activity. J Lipid Res. 60:783-793 (2019).

91. M. Jaye, K.J. Lynch, J. Krawiec, D. Marchadier, C. Maugeais, K. Doan, V. South, D. Amin, M. Perrone, and D.J. Rader. A novel endothelial-derived lipase that modulates HDL metabolism. Nat Genet. 21:424-428 (1999).

92. S.K. Koliwad, T. Kuo, L.E. Shipp, N.E. Gray, F. Backhed, A.Y. So, R.V. Farese, Jr., and J.C. Wang. Angiopoietin-like 4 (ANGPTL4, fasting-induced adipose factor) is a direct glucocorticoid receptor target and participates in glucocorticoid-regulated triglyceride metabolism. J Biol Chem. 284:25593-25601 (2009).

93. S. Kersten, L. Lichtenstein, E. Steenbergen, K. Mudde, H.F. Hendriks, M.K. Hesselink, P. Schrauwen, and M. Muller. Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via elevated free fatty acids. Arterioscler Thromb Vasc Biol. 29:969-974 (2009).

94. F. Dang, R. Wu, P. Wang, Y. Wu, M.S. Azam, Q. Xu, Y. Chen, and Y. Liu. Fasting and Feeding Signals Control the Oscillatory Expression of Angptl8 to Modulate Lipid Metabolism. Scientific reports. 6:36926 (2016).

95. Z. Fu, A.B. Abou-Samra, and R. Zhang. A lipasin/Angptl8 monoclonal antibody lowers mouse serum triglycerides involving increased postprandial activity of the cardiac lipoprotein lipase. Scientific reports. 5:18502 (2015).

96. W. Dijk, M. Heine, L. Vergnes, M.R. Boon, G. Schaart, M.K. Hesselink, K. Reue, W.D. van Marken Lichtenbelt, G. Olivecrona, P.C. Rensen, J. Heeren, and S. Kersten. ANGPTL4 mediates shuttling of lipid fuel to brown adipose tissue during sustained cold exposure. eLife. 4:(2015).

97. N.E. Gray, L.N. Lam, K. Yang, A.Y. Zhou, S. Koliwad, and J.C. Wang. Angiopoietin-like 4 (Angptl4) protein is a physiological mediator of intracellular lipolysis in murine adipocytes. J Biol Chem. 287:8444-8456 (2012).

98. M. Catoire, S. Alex, N. Paraskevopulos, F. Mattijssen, I. Evers-van Gogh, G. Schaart, J. Jeppesen, A. Kneppers, M. Mensink, P.J. Voshol, G. Olivecrona, N.S. Tan, M.K. Hesselink, J.F. Berbee, P.C. Rensen, E. Kalkhoven, P. Schrauwen, and S. Kersten. Fatty acid-inducible ANGPTL4 governs lipid metabolic response to exercise. Proc Natl Acad Sci U S A. 111:E1043-1052 (2014).

99. A.K. Singh, B. Aryal, B. Chaube, N. Rotllan, L. Varela, T.L. Horvath, Y. Suarez, and C. Fernandez-Hernando. Brown adipose tissue derived ANGPTL4 controls glucose and lipid metabolism and regulates thermogenesis. Molecular metabolism. 11:59-69 (2018).

100. R. Koishi, Y. Ando, M. Ono, M. Shimamura, H. Yasumo, T. Fujiwara, H. Horikoshi, and H. Furukawa. Angptl3 regulates lipid metabolism in mice. Nat Genet. 30:151-157 (2002).

101. K. Fujimoto, R. Koishi, T. Shimizugawa, and Y. Ando. Angptl3-null mice show low plasma lipid concentrations by enhanced lipoprotein lipase activity. Experimental animals. 55:27-34 (2006).

102. Y. Wang, M.C. McNutt, S. Banfi, M.G. Levin, W.L. Holland, V. Gusarova, J. Gromada, J.C. Cohen, and H.H. Hobbs. Hepatic ANGPTL3 regulates adipose tissue energy homeostasis. Proc Natl Acad Sci U S A. 112:11630-11635 (2015).

103. S. Banfi, V. Gusarova, J. Gromada, J.C. Cohen, and H.H. Hobbs. Increased thermogenesis by a noncanonical pathway in ANGPTL3/8-deficient mice. Proc Natl Acad Sci U S A. 115:E1249-E1258 (2018).

104. L.G.M. Janssen, M. Jauhiainen, V.M. Olkkonen, P.A.N. Haridas, K.J. Nahon, P.C.N. Rensen, and M.R. Boon. Short-Term Cooling Increases Plasma ANGPTL3 and ANGPTL8 in Young Healthy Lean Men but Not in Middle-Aged Men with Overweight and Prediabetes. Journal of clinical medicine. 8:(2019).

105. Y. Wang, V. Gusarova, S. Banfi, J. Gromada, J.C. Cohen, and H.H. Hobbs. Inactivation of ANGPTL3 reduces hepatic VLDL-triglyceride secretion. J Lipid Res. 56:1296-1307 (2015).

106. V. Gusarova, C.A. Alexa, Y. Wang, A. Rafique, J.H. Kim, D. Buckler, I.J. Mintah, L.M. Shihanian, J.C. Cohen, H.H. Hobbs, Y. Xin, D.M. Valenzuela, A.J. Murphy, G.D. Yancopoulos, and J. Gromada. ANGPTL3 blockade with a human monoclonal antibody reduces plasma lipids in dyslipidemic mice and monkeys. J Lipid Res. 56:1308-1317 (2015).

107. L. Lichtenstein, F. Mattijssen, N.J. de Wit, A. Georgiadi, G.J. Hooiveld, R. van der Meer, Y. He, L. Qi, A. Koster, J.T. Tamsma, N.S. Tan, M. Muller, and S. Kersten. Angptl4 protects against severe proinflammatory effects of saturated fat by inhibiting fatty acid uptake into mesenteric lymph node macrophages. Cell metabolism. 12:580-592 (2010).

108. P.J. Voshol, P.C. Rensen, K.W. van Dijk, J.A. Romijn, and L.M. Havekes. Effect of plasma triglyceride metabolism on lipid storage in adipose tissue: studies using genetically engineered mouse models. Biochimica et biophysica acta. 1791:479-485 (2009).

109. S. Romeo, L.A. Pennacchio, Y. Fu, E. Boerwinkle, A. Tybjaerg-Hansen, H.H. Hobbs, and J.C. Cohen. Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL. Nat Genet. 39:513-516 (2007).

110. D.I. Cho, H.J. Kang, J.H. Jeon, G.H. Eom, H.H. Cho, M.R. Kim, M. Cho, H.Y. Jeong, H.C. Cho, M.H. Hong, Y.S. Kim, and Y. Ahn. Antiinflammatory activity of ANGPTL4 facilitates macrophage polarization to induce cardiac repair. JCI insight. 4:(2019).

111. H. Okamoto, K. Cavino, E. Na, E. Krumm, S. Kim, P.E. Stevis, J. Harp, A.J. Murphy, G.D. Yancopoulos, and J. Gromada. Angptl4 does not control hyperglucagonemia or alpha-cell hyperplasia following glucagon receptor inhibition. Proc Natl Acad Sci U S A. 114:2747-2752 (2017).

112. A. Xu, M.C. Lam, K.W. Chan, Y. Wang, J. Zhang, R.L. Hoo, J.Y. Xu, B. Chen, W.S. Chow, A.W. Tso, and K.S. Lam. 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. 102:6086-6091 (2005).

113. Y. Wang, L.M. Liu, L. Wei, W.W. Ye, X.Y. Meng, F. Chen, Q. Xiao, J.Y. Chen, and Y. Zhou. Angiopoietin-like protein 4 improves glucose tolerance and insulin resistance but induces liver steatosis in high-fat-diet mice. Molecular medicine reports. 14:3293-3300 (2016).

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

115. V. Gusarova, C. O'Dushlaine, T.M. Teslovich, P.N. Benotti, T. Mirshahi, O. Gottesman, C.V. Van Hout, M.F. Murray, A. Mahajan, J.B. Nielsen, L. Fritsche, A.B. Wulff, D.F. Gudbjartsson, M. Sjogren, C.A. Emdin, R.A. Scott, W.J. Lee, A. Small, L.C. Kwee, O.P. Dwivedi, R.B. Prasad, S. Bruse, A.E. Lopez, J. Penn, A. Marcketta, J.B. Leader, C.D. Still, H.L. Kirchner, U.L. Mirshahi, A.H. Wardeh, C.M. Hartle, L. Habegger, S.N. Fetterolf, T. Tusie-Luna, A.P. Morris, H. Holm, V. Steinthorsdottir, P. Sulem, U. Thorsteinsdottir, J.I. Rotter, L.M. Chuang, S. Damrauer, D. Birtwell, C.M. Brummett, A.V. Khera, P. Natarajan, M. Orho-Melander, J. Flannick, L.A. Lotta, C.J. Willer, O.L. Holmen, M.D. Ritchie, D.H. Ledbetter, A.J. Murphy, I.B. Borecki, J.G. Reid, J.D. Overton, O. Hansson, L. Groop, S.H. Shah, W.E. Kraus, D.J. Rader, Y.I. Chen, K. Hveem, N.J. Wareham, S. Kathiresan, O. Melander, K. Stefansson, B.G. Nordestgaard, A. Tybjaerg-Hansen, G.R. Abecasis, D. Altshuler, J.C. Florez, M. Boehnke, M.I. McCarthy, G.D. Yancopoulos, D.J. Carey, A.R. Shuldiner, A. Baras, F.E. Dewey, and J. Gromada. Genetic inactivation of ANGPTL4 improves glucose homeostasis and is associated with reduced risk of diabetes. Nature communications. 9:2252 (2018).

116. I. Minicocci, A. Montali, M.R. Robciuc, F. Quagliarini, V. Censi, G. Labbadia, C. Gabiati, G. Pigna, M.L. Sepe, F. Pannozzo, D. Lutjohann, S. Fazio, M. Jauhiainen, C. Ehnholm, and M. Arca. Mutations in the ANGPTL3 gene and familial combined hypolipidemia: a clinical and biochemical characterization. The Journal of clinical endocrinology and metabolism. 97:E1266-1275 (2012).

117. K. Musunuru, J.P. Pirruccello, R. Do, G.M. Peloso, C. Guiducci, C. Sougnez, K.V. Garimella, S. Fisher, J. Abreu, A.J. Barry, T. Fennell, E. Banks, L. Ambrogio, K. Cibulskis, A. Kernytsky, E. Gonzalez, N. Rudzicz, J.C. Engert, M.A. DePristo, M.J. Daly, J.C. Cohen, H.H. Hobbs, D. Altshuler, G. Schonfeld, S.B. Gabriel, P. Yue, and S. Kathiresan. Exome sequencing, ANGPTL3 mutations, and familial combined hypolipidemia. N Engl J Med. 363:2220-2227 (2010).

118. D. Noto, A.B. Cefalu, V. Valenti, F. Fayer, E. Pinotti, M. Ditta, R. Spina, G. Vigna, P. Yue, S. Kathiresan, P. Tarugi, and M.R. Averna. Prevalence of ANGPTL3 and APOB gene mutations in subjects with combined hypolipidemia. Arterioscler Thromb Vasc Biol. 32:805-809 (2012).

119. A. Helgadottir, S. Gretarsdottir, G. Thorleifsson, E. Hjartarson, A. Sigurdsson, A. Magnusdottir, A. Jonasdottir, H. Kristjansson, P. Sulem, A. Oddsson, G. Sveinbjornsson, V. Steinthorsdottir, T. Rafnar, G. Masson, I. Jonsdottir, I. Olafsson, G.I. Eyjolfsson, O. Sigurdardottir, M.S. Daneshpour, D. Khalili, F. Azizi, D.W. Swinkels, L. Kiemeney, A.A. Quyyumi, A.I. Levey, R.S. Patel, S.S. Hayek, I.J. Gudmundsdottir, G. Thorgeirsson, U. Thorsteinsdottir, D.F. Gudbjartsson, H. Holm, and K. Stefansson. Variants with large effects on blood lipids and the role of cholesterol and triglycerides in coronary disease. Nat Genet. 48:634-639 (2016).

120. P. Tarugi, S. Bertolini, and S. Calandra. Angiopoietin-like protein 3 (ANGPTL3) deficiency and familial combined hypolipidemia. Journal of biomedical research. 33:73-81 (2019).

121. Y.X. Xu, V. Redon, H. Yu, W. Querbes, J. Pirruccello, A. Liebow, A. Deik, K. Trindade, X. Wang, K. Musunuru, C.B. Clish, C. Cowan, K. Fizgerald, D. Rader, and S. Kathiresan. Role of angiopoietin-like 3 (ANGPTL3) in regulating plasma level of low-density lipoprotein cholesterol. Atherosclerosis. 268:196-206 (2018).

122. T. Miidaand S. Hirayama. Impacts of angiopoietin-like proteins on lipoprotein metabolism and cardiovascular events. Curr Opin Lipidol. 21:70-75 (2010).

123. L. Pisciotta, E. Favari, L. Magnolo, S. Simonelli, M.P. Adorni, R. Sallo, T. Fancello, I. Zavaroni, D. Ardigo, F. Bernini, L. Calabresi, G. Franceschini, P. Tarugi, S. Calandra, and S. Bertolini. Characterization of three kindreds with familial combined hypolipidemia caused by loss-of-function mutations of ANGPTL3. Circulation Cardiovascular genetics. 5:42-50 (2012).

124. I. Minicocci, V. Cantisani, E. Poggiogalle, E. Favari, F. Zimetti, A. Montali, G. Labbadia, G. Pigna, F. Pannozzo, A. Zannella, F. Ceci, E. Ciociola, S. Santini, M. Maranghi, A. Vestri, P. Ricci, F. Bernini, and M. Arca. Functional and morphological vascular changes in subjects with familial combined hypolipidemia: an exploratory analysis. International journal of cardiology. 168:4375-4378 (2013).

125. L.Y. Yang, C.G. Yu, X.H. Wang, S.S. Yuan, L.J. Zhang, J.N. Lang, D. Zhao, and Y.M. Feng. Angiopoietin-Like Protein 4 Is a High-Density Lipoprotein (HDL) Component for HDL Metabolism and Function in Nondiabetic Participants and Type-2 Diabetic Patients. Journal of the American Heart Association. 6:(2017).

126. S. Kersten. New insights into angiopoietin-like proteins in lipid metabolism and cardiovascular disease risk. Curr Opin Lipidol. 30:205-211 (2019).

127. A.F. Labrijn, A.O. Buijsse, E.T. van den Bremer, A.Y. Verwilligen, W.K. Bleeker, S.J. Thorpe, J. Killestein, C.H. Polman, R.C. Aalberse, J. Schuurman, J.G. van de Winkel, and P.W. Parren. Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 in vivo. Nature biotechnology. 27:767-771 (2009).

128. M.G. Pouwer, E.J. Pieterman, N. Worms, N. Keijzer, J.W. Jukema, J. Gromada, V. Gusarova, and H.M.G. Princen. Alirocumab, evinacumab, and atorvastatin triple therapy regresses plaque lesions and improves lesion composition in mice. J Lipid Res(2019).

129. T.P. Prakash, J. Yu, M.T. Migawa, G.A. Kinberger, W.B. Wan, M.E. Ostergaard, R.L. Carty, G. Vasquez, A. Low, A. Chappell, K. Schmidt, M. Aghajan, J. Crosby, H.M. Murray, S.L. Booten, J. Hsiao, A. Soriano, T. Machemer, P. Cauntay, S.A. Burel, S.F. Murray, H. Gaus, M.J. Graham, E.E. Swayze, and P.P. Seth. Comprehensive Structure-Activity Relationship of Triantennary N-Acetylgalactosamine Conjugated Antisense Oligonucleotides for Targeted Delivery to Hepatocytes. Journal of medicinal chemistry. 59:2718-2733 (2016).

130. S.T. Crooke, S. Wang, T.A. Vickers, W. Shen, and X.H. Liang. Cellular uptake and trafficking of antisense oligonucleotides. Nature biotechnology. 35:230-237 (2017).

131. M.J. Graham, R.G. Lee, T.A. Brandt, L.J. Tai, W. Fu, R. Peralta, R. Yu, E. Hurh, E. Paz, B.W. McEvoy, B.F. Baker, N.C. Pham, A. Digenio, S.G. Hughes, R.S. Geary, J.L. Witztum, R.M. Crooke, and S. Tsimikas. Cardiovascular and Metabolic Effects of ANGPTL3 Antisense Oligonucleotides. N Engl J Med. 377:222-232 (2017).

132. U. Desai, E.C. Lee, K. Chung, C. Gao, J. Gay, B. Key, G. Hansen, D. Machajewski, K.A. Platt, A.T. Sands, M. Schneider, I. Van Sligtenhorst, A. Suwanichkul, P. Vogel, N. Wilganowski, J. Wingert, B.P. Zambrowicz, G. Landes, and D.R. Powell. Lipid-lowering effects of anti-angiopoietin-like 4 antibody recapitulate the lipid phenotype found in angiopoietin-like 4 knockout mice. Proc Natl Acad Sci U S A. 104:11766-11771 (2007).

133. H. Ge, G. Yang, X. Yu, T. Pourbahrami, and C. Li. Oligomerization state-dependent hyperlipidemic effect of angiopoietin-like protein 4. J Lipid Res. 45:2071-2079 (2004).

134. A.C. Chadwick, N.H. Evitt, W. Lv, and K. Musunuru. Reduced Blood Lipid Levels With In Vivo CRISPR-Cas9 Base Editing of ANGPTL3. Circulation. 137:975-977 (2018).

135. X. Wangand K. Musunuru. Angiopoietin-Like 3: From Discovery to Therapeutic Gene Editing. JACC Basic to translational science. 4:755-762 (2019).

136. R. Dunbar, A. Bouzelmat, G. D.A., R. Pordy, W. Sasiela, S. Mellis, and P. Banerjee. Abstract 19133: Inhibition of ANGPTL3 by Evinacumab Reduced Triglycerides (TGs) and LDL-C in Subjects Presenting With Modest Elevations in TGs and/or LDL-C, Recapitulating the Hypolipidemic Effects of Loss-of-Function (LoF) Mutations of ANGPTL3. Circulation. 134:A19133 (2016).

137. Z. Ahmad, P. Banerjee, S. Hamon, K.C. Chan, A. Bouzelmat, W.J. Sasiela, R. Pordy, S. Mellis, H. Dansky, D.A. Gipe, and R.L. Dunbar. Inhibition of Angiopoietin-Like Protein 3 With a Monoclonal Antibody Reduces Triglycerides in Hypertriglyceridemia. Circulation(2019).

138. B.G. Nordestgaard, M. Benn, P. Schnohr, and A. Tybjaerg-Hansen. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA. 298:299-308 (2007).

139. Y.I. Miller, S.H. Choi, L. Fang, and S. Tsimikas. Lipoprotein modification and macrophage uptake: role of pathologic cholesterol transport in atherogenesis. Sub-cellular biochemistry. 51:229-251 (2010).

140. G.F. Watts, E.M. Ooi, and D.C. Chan. Demystifying the management of hypertriglyceridaemia. Nature reviews Cardiology. 10:648-661 (2013).

141. M. Ruscica, C. Macchi, F. Fogacci, N. Ferri, E. Grandi, E. Rizzoli, S. D'Addato, C. Borghi, A.F. Cicero, and G. Brisighella Heart Study. Angiopoietin-like 3 and subclinical peripheral arterial disease: Evidence from the Brisighella Heart Study. European journal of preventive cardiology:2047487319884378 (2019).

142. I. Goldenberg, M. Benderly, R. Sidi, V. Boyko, A. Tenenbaum, D. Tanne, and S. Behar. Relation of clinical benefit of raising high-density lipoprotein cholesterol to serum levels of low-density lipoprotein cholesterol in patients with coronary heart disease (from the Bezafibrate Infarction Prevention Trial). Am J Cardiol. 103:41-45 (2009).

143. N. Ferri, A. Corsini, C. Sirtori, and M. Ruscica. PPAR-alpha agonists are still on the rise: an update on clinical and experimental findings. Expert opinion on investigational drugs. 26:593-602 (2017).

144. L.E. Simental-Mendia, M. Simental-Mendia, A. Sanchez-Garcia, M. Banach, S.L. Atkin, A.M. Gotto, Jr., and A. Sahebkar. Effect of fibrates on glycemic parameters: A systematic review and meta-analysis of randomized placebo-controlled trials. Pharmacological research. 132:232-241 (2018).

145. D.L. Bhatt, P.G. Steg, M. Miller, E.A. Brinton, T.A. Jacobson, S.B. Ketchum, R.T. Doyle, Jr., R.A. Juliano, L. Jiao, C. Granowitz, J.C. Tardif, C.M. Ballantyne, and R.-I. Investigators. Cardiovascular Risk Reduction with Icosapent Ethyl for Hypertriglyceridemia. N Engl J Med. 380:11-22 (2019).

146. D.L. Bhatt, P.G. Steg, M. Miller, E.A. Brinton, T.A. Jacobson, S.B. Ketchum, R.T. Doyle, Jr., R.A. Juliano, L. Jiao, C. Granowitz, J.C. Tardif, J. Gregson, S.J. Pocock, C.M. Ballantyne, and R.-I. Investigators. Effects of Icosapent Ethyl on Total Ischemic Events: From REDUCE-IT. J Am Coll Cardiol. 73:2791-2802 (2019).

147. G. Kolovou, O. Diakoumakou, V. Kolovou, E. Fountas, S. Stratakis, E. Zacharis, E.N. Liberopoulos, F. Matsouka, A. Tsoutsinos, I. Mastorakou, T. Katsikas, S. Mavrogeni, and G. Hatzigeorgiou. Microsomal triglyceride transfer protein inhibitor (lomitapide) efficacy in the treatment of patients with homozygous familial hypercholesterolaemia. European journal of preventive cardiology:2047487319870007 (2019).

148. F.M. Sacks, M. Stanesa, and R.A. Hegele. Severe hypertriglyceridemia with pancreatitis: thirteen years' treatment with lomitapide. JAMA internal medicine. 174:443-447 (2014).

149. C. Macchi, C.R. Sirtori, A. Corsini, R.D. Santos, G.F. Watts, and M. Ruscica. A New Dawn for Managing Dyslipidemias: The Era of Rna-Based Therapies. Pharmacological research:104413 (2019).

150. J.L. Witztum, D. Gaudet, S.D. Freedman, V.J. Alexander, A. Digenio, K.R. Williams, Q. Yang, S.G. Hughes, R.S. Geary, M. Arca, E.S.G. Stroes, J. Bergeron, H. Soran, F. Civeira, L. Hemphill, S. Tsimikas, D.J. Blom, L. O'Dea, and E. Bruckert. Volanesorsen and Triglyceride Levels in Familial Chylomicronemia Syndrome. N Engl J Med. 381:531-542 (2019).

## Figure legends

**Figure 1. Schematic representation of ANGPTL3, ANGPTL4 and ANGPTL8 protein structures.** ANGPTL3 (Angiopoietin-like protein 3) is composed by an N-terminal domain involved in the inhibition of lipoprotein lipase (LPL) and endothelial lipase and by a C-terminal fibrinogen-like domain. ANGPTL4 shares with ANGPTL3 both the coiled-coil domain and the fibrinogen-like domain (FLD). ANGPTL8 is paralog of the N-terminal region of ANGPTL3 and it is required for ANGPTL3 activation. All isoforms contain a Specific Epitope1 (SE1) required to inhibit LPL activity. LR stands for linker region. Modified from Lupo et al. (49)

**Figure 2. Biology and pharmacological inhibition of ANGPTL3 and ANGPTL4.** ANGPTL3 (Angiopoietin-like protein 3) is secreted by hepatocytes and inhibits lipoprotein lipase (LPL) in peripheral tissues, such as skeletal muscle and adipose tissues. Differently, ANGPTL4 is induced by fasting or physical exercise; it is released by the liver, the skeletal muscle and the white adipose tissue. ANGPTL3 and ANGPTL4 inhibit LPL and endothelial lipase (EL), thus increasing the levels of TG-rich-lipoproteins, FFA and glycerol. Phase I clinical trials with monoclonal antibodies (evinacumab) and antisense oligonucleotide (ASO) against anti-ANGPTL3 are ongoing, while an antibody anti-ANGPTL4, 14D12, is still in preclinical development. ER: endoplasmic reticulum; FFA, free fatty acids; HDL, high-density lipoprotein; TG, triglycerides; TGRL, triglyceride-rich lipoproteins.

**Table 1.** Effect of ANGPTL3 or ANGPTL4 inhibition on lipid profile in pre-clinical studies.

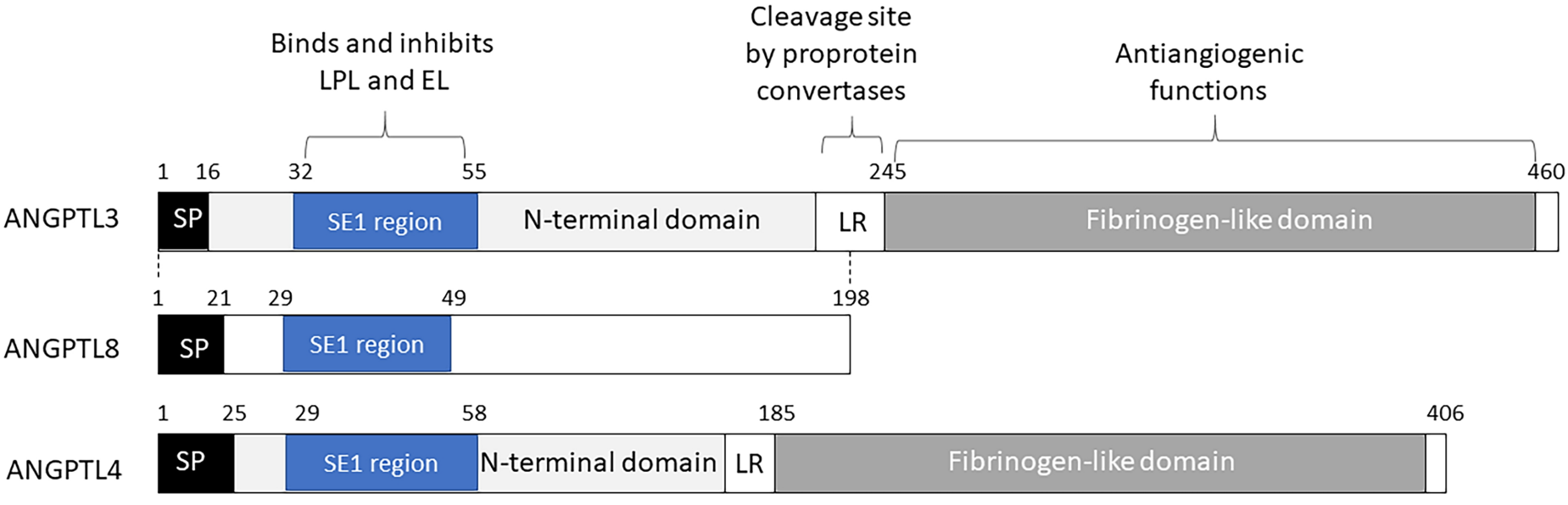
| Treatment | Model | Efficacy |
|---|---|---|
| Evinacumab | Wild type and LDLr$^{-/-}$ mice (105) | TG -40%; TC -33%; reduced apoB, increased clearance of apoB-containing lipoproteins |
| | Wild type mice fed High fat diet (106) | TG -53%; TC -35%; LDL-C -45% |
| | Spontaneous hypertriglyceridemic Cynomolgus monkey (106) | TG -48% (3mg/Kg) /-89% (10mg/Kg); non-HDL-C -44%; LDL-C: no changes |
| | ApoE3-Leiden CETP mice fed Western diet (128) | In combination with alirocumab and atorvastatin: TG -67%; TC -68%; non-HDL-C -84% compared to atorvastatin; stronger regression of atherosclerosis lesion size, improved plaque composition compared to control |
| *Angptl3* ASO | (i) LDLr $^{-/-}$ and apoC-III $^{-/-}$ mice fed chow and high fat diets<br>(ii) mice expressing human apoC-III (13) | TG -35/-85%; LDL-C -7/-64%; delayed *en face* atherosclerosis progression in LDLr$^{-/-}$ mice<br>Fed Western-diet |
| Angptl3 CRISPR-Cas9 base editing | Wild type mice (134) | TG -31%, TC -19% |
| | Hyperlipidemic LDL$^{-/-}$ mice (134) | TG -56%, TC -51% |
| anti-Angptl4 mAb 14D12 | Wild type mice fed chow and high fat diets (132) | TG:-50/-59%; TC ≈-30%; rised TG clearance; reduced VLDL production |
| | apoE$^{-/-}$, LDLr$^{-/-}$ and db/db mice (132) | TG ≈ -55%; TC ≈ -25% in LDLr-/- and db/db mice, no changes in apoE$^{-/-}$ mice |

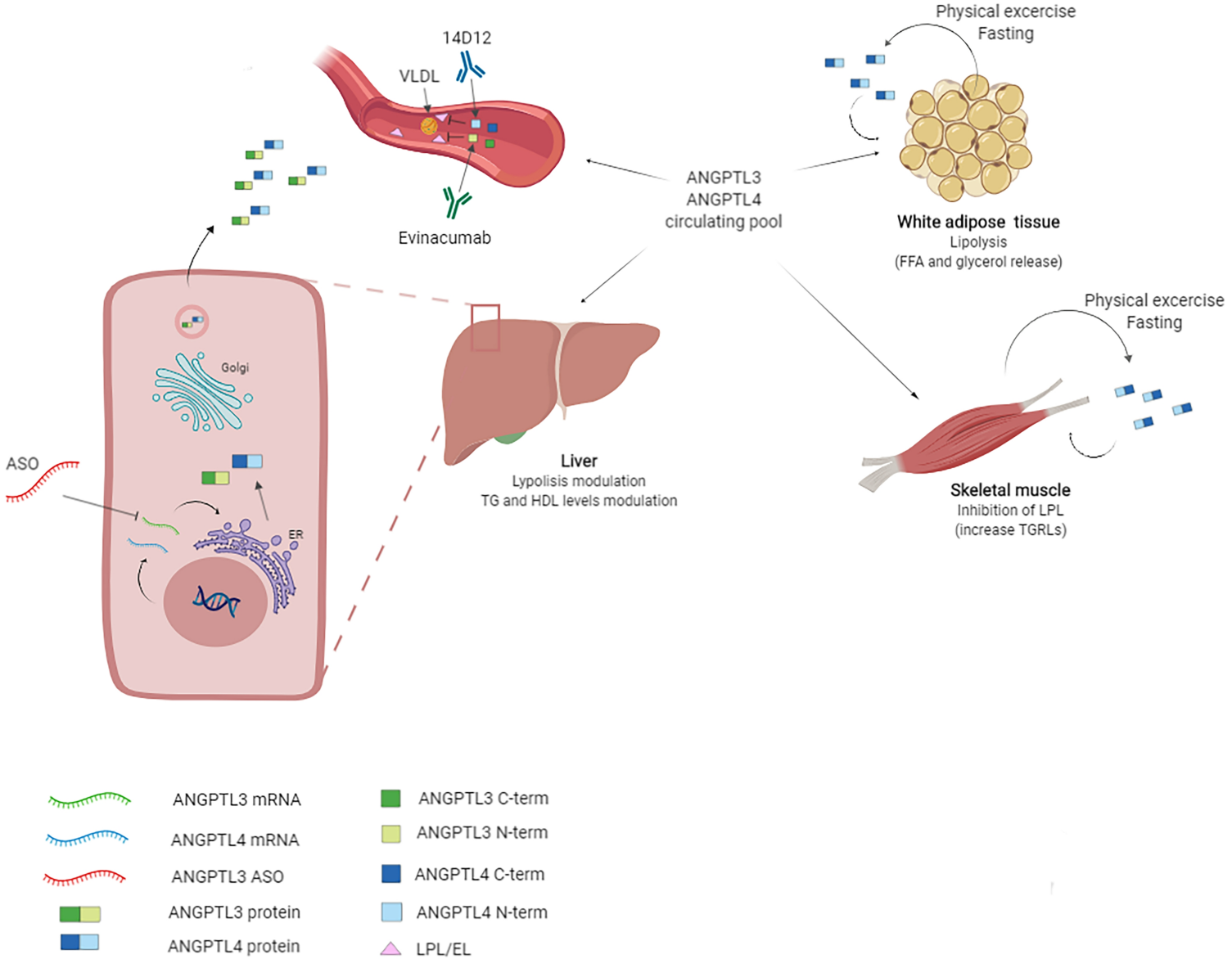
Angptl3, angiopoietin like 3; apoB, apolipoprotein B; apoE, apolipoprotein E; ASO, Antisense Oligonucleotide; CETP, cholesteryl transfer protein; HDL, high density lipoproteins; LDL, low density lipoproteins; TC, total cholesterol; TG, triglycerides; VLDL, very low-density lipoproteins

**Table 2.** Effect of ANGPTL3 inhibition on lipid profile in clinical studies.

| Treatment | Dose | Efficacy |
|---|---|---|
| Evinacumab in subjects with 150≤ TG ≤450 mg/dL (136) | Ascending single-dose 75, 150, 250 mg for s.c. or 5, 10, 20 mg/Kg for i.v. | TG -1% (lowest dose) to -75% (highest dose)<br>LDL-C -3.4% (lowest dose) to -25.5% (highest dose) |
| | Ascending single-dose 75, 150, 250 mg for s.c. or 5, 10, 20 mg/Kg for i.v. | TG maximum reduction of 76.9% with 10 mg/kg i.v. |
| Evinacumab in subjects with 150≤ TG ≤450 mg/dL (137) | Multiple ascending dose 150/300/450 mg once weekly, 300/450 mg every 2 weeks for s.c. or 20 mg/kg once every 4 weeks i.v. | TG maximum reduction of 83.1% with 20 mg/kg i.v. once every 4 weeks<br>. |
| Evinacumab in adults with homozygous familial hypercholesterolemia (39) | 250 mg s.c. at baseline and 15 mg/kg i.v. at week 2 | LDL-C -49% (range, 25 to 90)<br>TG -47% (interquartile range, 38 to 57) |
| ANGPTL3-$L_{Rx}$ in healthy volunteers aged 18-65 years with TG>150 mg/dL (131) | Multiple-ascending dose 10, 20, 40 or 60 mg per week for 6 weeks | TG from -33.2% to -63.1%,<br>LDL-C from -1.3% to -32.9%<br>VLDL-C from -27.9% to -60% |

ANGPTL3, angiopoietin like 3; i.v., intravenous; LDL, low density lipoproteins; TC, total cholesterol; TG, triglycerides; s.c., subcutaneous; VLDL, very low-density lipoproteins


Binds and inhibits
LPL and EL
Cleavage site
by proprotein
convertases
Antiangiogenic
functions
1
16
32
55
245
460
ANGPTL3
SP
SE1 region
N-terminal domain
LR
Fibrinogen-like domain
1
21
29
49
198
ANGPTL8
SP
SE1 region
1
25
29
58
185
406
ANGPTL4
SP
SE1 region
N-terminal domain
LR
Fibrinogen-like domain


14D12
VLDL
Evinacumab
Physical excercise
Fasting
ANGPTL3
ANGPTL4
circulating pool
White adipose tissue
Lipolysis
(FFA and glycerol release)
Golgi
ASO
ER
Liver
Lypolisis modulation
TG and HDL levels modulation
Physical excercise
Fasting
Skeletal muscle
Inhibition of LPL
(increase TGRLs)
ANGPTL3 mRNA
ANGPTL4 mRNA
ANGPTL3 ASO
ANGPTL3 protein
ANGPTL4 protein
ANGPTL3 C-term
ANGPTL3 N-term
ANGPTL4 C-term
ANGPTL4 N-term
LPL/EL

Relative to the manuscript “Pharmacological aspects of ANGPTL3 and AGPTL4 inhibitors: new therapeutic approaches for the treatment of atherogenic dyslipidemia” the Authors declare no conflict of interests.