CHAPTER 1

# General Introduction

Cardiovascular disease (CVD) causes a global burden with a death rate of 17.3 million per year which is rapidly inclining to an estimated 23.6 million in 2030.[1] Atherosclerosis, a chronic inflammatory disease of multifactorial origin that may ultimately lead to stenosis or atherothrombosis,[2, 3] is a dominant contributor to the development of CVD.[4] It is characterized by the development of atherosclerotic lesions consisting of activated endothelial cells, lipid accumulation, leukocytes, macrophages, foam cells, connective-tissue elements, calcified regions and necrotic cores.[3, 5] The progressive decrease in lumen size caused by the development of these lesions was previously described as the culprit that led to cardiovascular events.[3] However, it is now believed that this is attributable to a decrease in plaque stability, which may lead to rupture followed by thrombus formation on the ruptured plaques.[6] To this end, an unstable lesion is characterized by a thin, collagen-poor fibrous cap, decreased smooth muscle cells, increased macrophage infiltration and a large necrotic core.[5] This type of vulnerable lesion is referred to as a thin-cap fibroatheroma.[7] From a pharmaceutical perspective, several risk factors are currently being targeted in the fight against the development of atherosclerosis and the prevalence of CVD. These include amongst others; hypertension and high blood cholesterol, more specifically high low-density lipoprotein-cholesterol (LDL-C) and low high-density lipoprotein-cholesterol (HDL-C).[8]

## 1. Hypertension

Uncontrolled hypertension is the leading risk factor for CVD.[1] Numerous factors including; age, ethnicity, family history, genetic factors, lower education and socioeconomic status, obesity, smoking, sleep apnea and dietary factors contribute to the development of hypertension and several of these factors are modifiable. Nonetheless, according to statistics from the American Heart Association, the prevalence of high blood pressure, defined as a systolic blood pressure of ≥140 mmHg and a diastolic blood pressure of ≥90 mmHg, or taking anti-hypertensive medication, or being diagnosed with hypertension on at least two occasions, was as high as 33% for adults aged ≥20 years (extrapolated to 2010 using data from the National Health and Nutrition Examination Survey (NHANES) 2007 to 2010).[8] The World Health Organization reported that globally elevated blood pressure caused 51% of stroke deaths and 45% of coronary heart disease (CHD) deaths.[1] The inefficiency of the current treatment regimens to reduce these numbers is ascribed not only to lack of adherence, but also to failure of treatment strategies to fully neutralize all mechanisms involved in hypertension, as well as the activation of feedback mechanisms that counteract the treatment effects on blood pressure.[9] Therefore, the development of additional anti-hypertensive treatment options beyond the current 'gold standard' therapies, such as selective calcium channel blockers, β-blockers, diuretics, angiotensin converting enzyme

inhibitors (ACEi) and angiotensin II type I receptor blockers (ARBs),[10] is needed to more effectively treat hypertension.

The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in the regulation of blood pressure. Renin secreted by the kidneys cleaves angiotensinogen produced by the liver to angiotensin I which is converted by angiotensin converting enzyme to angiotensin II. Angiotensin II, in turn binds to angiotensin II receptors leading to arterial vasoconstriction, other tubular and glomerular effects, as well as inflammation, hypertrophy and fibrosis.[10] Current treatment strategies that disrupt the RAAS, such as ACEi and ARBs result in compensatory increases in angiotensin I or II, as well as in plasma renin activity (PRA).[11] The latter may have further implications since renin was shown to exert angiotensin I-independent effects by binding to (pro)renin receptors.[10] Moreover, increased PRA has been associated with increased mortality rates as a result of myocardial infarction (MI) and renal failure.[12] The development of direct renin inhibitors emerged as a potential treatment strategy to more effectively inhibit the RAAS at the point of origin and at its rate-limiting step.[13] Aliskiren is the first orally active direct renin inhibitor approved for the treatment of hypertension.[13] The extent to which aliskiren, administered as monotreatment and in combination with current 'gold standard' treatment strategies in various patient populations, can provide clinical benefit remains to be elucidated.

## 2. High blood cholesterol

Cholesterol is a hydrophobic molecule that serves as a structural component in plasma membranes and as a precursor for the synthesis of steroid hormones and bile acids.[14, 15] Cholesterol is transported through the circulation in five major classes of lipoprotein particles; chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), LDL and HDL.[15] Chylomicrons transport dietary lipids after uptake and secretion from the intestines and VLDL is secreted from the liver to deliver triglycerides and cholesterol to other tissues.

### 2.1 Low-density lipoprotein-cholesterol

In 1913, Nikolai N. Anitschkow first described the involvement of cholesterol in the development of atherosclerosis when rabbits fed a high-cholesterol diet developed human-like arterial lesions.[16] The recent 100$^{th}$ year anniversary of this discovery is worth commemorating given that serum cholesterol contained in LDL particles is now well recognized as a primary causal risk factor for CHD as evidenced by experimental, epidemiological and genetic studies.[17]

The American Heart Association reported a prevalence of hypercholesterolemia defined by TC levels ≥200 mg/dL of 43.4% and by LDL-C levels ≥130 mg/dL of 31.1% (extrapolated to 2010 using data from NHANES 2007 to 2010).[8] The prevalence of high TC and LDL-C levels in 2009/2010 was considerably lower compared to 1999/2000, most likely attributable to statin use.[1] Statins reduce LDL-C up to 55% by inhibiting hydroxy-3-methyl-glutaryl-CoA reductase, a rate limiting step in cholesterol biosynthesis.[18] Intervention trials provided ample evidence that the lowering of LDL-C with statin therapy contributes to a reduction of CHD[19-21] and recent trials indicated that intensive lipid-lowering with statins may be more beneficial in risk reduction than less intensive (or standard) therapy.[19] According to results from the latter meta-analysis, every 1 mmol/L (40 mg/dL) reduction in LDL-C was associated with a 22% reduction in the risk of major vascular events, suggesting that a 2-3 mmol/L reduction in LDL-cholesterol (LDL-C) would correspond with a 40-50% reduction in events. Nonetheless, there remains a substantial residual risk despite statin treatment which warrants the development of other treatment options to better protect against CVD, especially in combination with statins.

### *2.1.1 Approved LDL-C-lowering treatment strategies beyond statins*

Despite not sharing the success rate of statin treatment, other LDL-C-lowering treatment strategies have been approved for clinical use. These include: bile acid-binding resins, cholesterol absorption inhibitors, niacin, peroxisome proliferator-activated receptor (PPAR)-α agonists and PPAR-γ agonists.[17] In fact, recently the cholesterol absorption inhibitor, ezetimibe was the first compound shown to add to the effect of a statin on CVD outcome (http://newsroom.heart.org/news/cholesterol-lowering-drug-with-different-action-adds-to-statins-reduction-of-cardiovascular-risk).

Bile acid-binding resins and cholesterol absorption inhibitors were developed to inhibit cholesterol absorption in the intestine from food and bile.[22] Resins reduce the efficiency of cholesterol absorption by binding bile acids and therewith decreasing intestinal solubilization of lipids, and by binding bile acids resins also increase bile acid synthesis from the precursor cholesterol.[23] The cholesterol absorption inhibitor, ezetimibe limits cholesterol absorption by blocking the function of the transporter Niemann pick C-1-like 1 (NPC1L1). The benefits of niacin on plasma lipids were first reported in 1955 and led to the development of niacin for therapeutic purposes.[24] The lipid-lowering effects of niacin is ascribed to decreased free fatty acid (FFA) flux from adipose tissue to the liver, although this reduction in FFAs is followed by a rebound effect. Another mechanism described by which niacin decreases lipids is by decreasing TG synthesis.[25] PPARs are nuclear transcription factors involved in the regulation of target gene expression and their effects on glucose and lipid metabolism were utilized to develop PPAR agonists for the treatment of hyperglycemia and dyslipidemia.[26, 27] PPAR-α activation decreases lipids by increasing lipoprotein lipase-mediated lipolysis,

VLDL remnants clearance and β-oxidation.[28] PPAR-γ agonists mainly mediate glucose homeostasis,[26] but pioglitazone also weakly activates PPAR-α and, therefore, also has minor effects on lipid metabolism.[29]

### *2.1.2. Emerging LDL-C-lowering treatment strategies beyond statins*

Several other approaches to lower LDL-C are currently being investigated in clinical trials, including amongst others: apolipoprotein B inhibition by for example antisense oligonucleotides (ASOs), microsomal triglyceride transport protein (MTP) inhibitors, proprotein convertase subtilisin kexin type 9 (PCSK9) inhibition by for instance monoclonal antibodies, gene-silencing or vaccines and PPAR-δ agonists.[17] Some of these treatment strategies have already been approved in certain countries.

The ASO against apolipoprotein B, mipomersen inhibits the synthesis of apolipoprotein B by binding to the messenger RNA coding for apolipoprotein B-100 and the MTP inhibitor, lomitapide inhibits the transfer of triglycerides to apolipoprotein B during formation of a mature VLDL particle within hepatocytes. Both compounds, therefore, decrease LDL-C by reducing VLDL production and secretion.[17] PCSK9 is a serine protease responsible for LDL receptor (LDLR) degradation.[30] Interestingly, the upregulation of the LDLR after statin treatment is accompanied by an upregulation of PCSK9 which in turn promotes LDLR degradation.[31-33] PCSK9 inhibition has, therefore, emerged as a promising new strategy to lower LDL-C, especially in combination with statins. PPAR-δ agonists also improve atherogenic lipid profiles by modifying cell fuel preference from glucose to lipids[34] and a reduction in cholesterol absorption via NPC1L1 has been described as another possible mechanism.[35]

## 2.2 High-density lipoprotein-cholesterol

In the 1970s, Miller & Miller hypothesized that a reduction in plasma HDL concentration may accelerate the development of atherosclerosis and ischemic heart disease by impairing cholesterol clearance from the arterial wall.[36] Besides its major role in reverse cholesterol transport, HDL has also been described to have anti-inflammatory, anti-oxidant, anti-platelet and vasodilatory properties.[37] Although the original hypothesis referred to HDL particle concentration which could not be measured at the time,[37] epidemiological studies consistently reported an inverse association between CHD risk and HDL-C.[38-40] Results from 4 prospective epidemiologic studies indicated that an increase of 1 mg/dL (0.03 mM) in HDL-C was associated with a 2-3% reduction in CHD risk.[41] However, data from genetic studies do not support a causal relationship between increased HDL-C and reduced risk of MI[42, 43] and evidence from large clinical trials is lacking.

The American Heart Association revealed a prevalence of HDL-C levels ≤40 mg/dL of 21.8% (extrapolated to 2010 using data from NHANES 2007 to 2010). Whereas currently

no clinical trial has demonstrated beneficial effects of HDL-C-raising therapies, several HDL-targeting therapies are still being investigated in clinical trials.

#### *2.2.1. HDL-C-raising treatment strategies beyond statins*

Treatment strategies approved for the treatment of hyperlipidemia, such as niacin and PPAR-α agonists (fibrates), that mainly decreases triglycerides with a small LDL-C-lowering effect, also increases HDL-C. Other therapies in clinical development primarily aimed to increase HDL-C include CETP inhibitors, scavenger receptor B-I (SR-BI) inhibitors and apolipoprotein A-I inducers.[44] In addition, the effects of novel treatment strategies specifically targeting HDL are currently being investigated in clinical trials, including reconstituted and delipidated HDL, as well as HDL mimetics, apolipoprotein A-I mimetic peptides and recombinant human lecithin cholesterol acyltransferase (LCAT).

Niacin is described to increase HDL-C by increasing apolipoprotein A-I lipidation and by decreasing apolipoprotein A-I removal,[25, 45] and PPAR-α agonists are shown to increase HDL-C by increasing apolipoprotein A-I/II expression and cholesterol efflux from macrophages.[26] In 1989, markedly increased HDL-C led to the discovery of the first mutation in the CETP gene in two Japanese subjects.[46] CETP facilitates the transfer of cholesteryl esters from atheroprotective HDL to atherogenic (V)LDL and has become a target to increase HDL-C.[47] The HDL-C-raising effects of niacin and PPAR-α agonists are also ascribed to a reduction in CETP.[48, 49] The PPAR-γ agonist, pioglitazone increases HDL-C by weakly activating PPAR-α.[29] PPAR-δ agonists also increase HDL-C and this effect is ascribed to possible mechanisms involving apolipoprotein A-II and ABCA1.[35] The development of reconstituted and delipidated HDL, as well as HDL mimetics, apolipoprotein A-I mimetic peptides and recombinant human lecithin cholesterol acyltransferase (LCAT) have emerged as potential approaches to improve reverse cholesterol transport.[50] In addition, the therapeutic use of recombinant apolipoprotein A-I Milano originated from the observation that carriers of this mutation have low levels of HDL-C without increased atherosclerosis as observed in patients with hypoalhalipoproteinemia.[51, 52] It remains to be elucidated whether these novel treatment strategies may provide additional clinical benefit beyond current therapies.

## 3. Experimental model for human-like lipoprotein metabolism and atherosclerosis

To investigate the effects of innovative pharmaceutical interventions in experimental atherosclerosis, we used the APOE*3Leiden.CETP mouse model. While normal wild-type mice have a very rapid clearance of apolipoprotein B-containing lipoproteins, APOE*3Leiden.CETP mice have impaired clearance of apolipoprotein B-containing lipoproteins and

mimic the slow clearance observed in humans, particularly in patients with familial dysbetalipoproteinemia (FD).[53] The APOE*3Leiden mouse was initially developed as an animal model for FD or type III hyperlipoproteinemia, which is characterized by elevated levels of cholesterol and an increased ratio of cholesterol to triglycerides in the VLDL and IDL fractions, resulting in the appearance of β-VLDL particles.[53, 54] Similar to FD patients, APOE*3Leiden and APOE*3Leiden.CETP mice carry a major part of plasma cholesterol in the VLDL and VLDL-remnant particles, leading to the formation of β-VLDL particles, which further increases after cholesterol feeding. These mice respond in a similar way to statins as humans with decreases in the apolipoprotein B-containing lipoproteins up to 55%. In addition, APOE*3Leiden.CETP mice express human CETP under control of its natural flanking regions,[55] a crucial gene involved in HDL metabolism and implicated in the mechanisms by which most therapies modulate HDL.[49] These mice develop diet-induced atherosclerosis and respond to TC/LDL-C-lowering and HDL-C-raising drugs in a human-like manner[45, 48, 56-58] and are, therefore, a suitable model to study the effects of innovative pharmaceutical interventions on lipid and lipoprotein metabolism and atherosclerosis development.

## 4. Outline of this thesis

The research described in this thesis investigated the effects of innovative pharmaceutical interventions in experimental atherosclerosis. Statin treatment is currently the first line of defense against CVD. However, the treatment of CVD remains suboptimal due to; (i) a residual risk that persists after statin treatment, (ii) failure for some patients to reach LDL-C targets despite statin treatment, and (iii) lack of adherence to statin treatment as a result of statin intolerance. We, therefore investigated the effects of novel treatment strategies administered as monotreatment, but also in combination with statin treatment on atherosclerosis development in APOE*3.Leiden.CETP mice, a well-established model for lipid metabolism and atherosclerosis.

Hypertension is a leading risk factor for CVD and is associated with the development of atherosclerosis. Aliskiren is the first commercially available, orally active, direct renin inhibitor approved for the treatment of hypertension. In **chapter 2**, we investigated the effects of aliskiren administered as monotreatment and in combination with atorvastatin on systolic blood pressure, total cholesterol, inflammation markers and atherosclerotic lesion size and composition in APOE*3Leiden.CETP mice.

Cholesterol contained in LDL particles is well recognized as a primary causal risk factor for CHD as evidenced by experimental, epidemiological and genetic data. Furthermore, intervention trials provided ample evidence that the lowering of LDL-C contributes to a reduction in CHD. However, despite the fact that epidemiological studies consistently

reported an inverse association between HDL-C and CHD risk, the benefits of raising HDL-C remain less defined. In chapter 3 to 6, we investigated the effects of novel lipid-modifying treatment strategies, i.e. LDL-C lowering and/or HDL-C-raising compounds on atherosclerosis development in the APOE*3Leiden.CETP mouse model, since these mice respond to both LDL-C-lowering and HDL-C-raising compounds in a human-like manner.

The benefits of niacin on plasma lipids were first described in 1955 and led to the development of niacin for therapeutic purposes. In **chapter 3**, we evaluated the effects of niacin alone and in combination with simvastatin on plasma lipid levels and atherosclerotic lesion size and composition. To further explore the mechanism by which niacin reduces atherosclerosis, we performed additional VLDL production and clearance, as well as reverse cholesterol transport experiments. We also conducted statistical analyses to assess the contribution of the LDL-C-lowering versus HDL-C-raising effects of niacin on the inhibition of atherosclerosis.

CETP is involved in lipoprotein metabolism by facilitating the transfer of cholesterol esters from atheroprotective HDL to atherogenic (V)LDL. In **chapter 4**, we investigated the effects of a broad dose range of the novel CETP inhibitor anacetrapib on CETP activity, lipid levels, atherosclerotic lesion size and composition and HDL function. In addition, we examined possible additive/synergistic effects of anacetrapib on top of atorvastatin. We also performed statistical analyses to evaluate whether the effects of anacetrapib and atorvastatin on atherosclerosis development could be explained by either a decrease in non-HDL-C or an increase in HDL-C or both. Since lowering of non-HDL-C was a major determinant of lesion size, we investigated the mechanism by which anacetrapib decreases (V)LDL-C levels in **chapter 5**.

PCSK9 is a serine protease responsible for LDLR degradation. The upregulation of the LDLR after statin treatment is accompanied by an upregulation of PCSK9 which in turn promotes LDLR degradation. Inhibition of PCSK9 is, therefore, a potential novel strategy in the treatment against CVD, especially in combination with statin treatment. In **chapter 6**, we investigated the effects of 2 dosages of the fully human, monoclonal antibody, alirocumab alone and in combination with atorvastatin on hepatic LDLR protein levels, hepatic and plasma lipid levels, atherosclerosis development and plaque morphology.

In **chapter 7**, we reviewed the effects of established and novel treatment strategies, specifically targeting HDL, on inhibition of atherosclerosis development in animals expressing CETP, a crucial gene involved in HDL metabolism and implicated in the mechanisms by which most therapies modulate HDL. In addition, we conducted a meta-analysis to evaluate the potential effects of these treatment strategies on the prevention of clinical events in randomized controlled trials. In this systematic review and meta-analysis of preclinical studies and clinical trials, we focused specifically on the contribution of non-HDL-C/LDL-C-lowering versus HDL-C-raising on inhibition of atherosclerosis and the prevention of CVD.

The results obtained in these studies and their clinical relevance are discussed in the General discussion and future perspectives in **chapter 8**.

## References


1. Laslett LJ, Alagona P, Jr., Clark BA, 3rd, Drozda JP, Jr., Saldivar F, Wilson SR, Poe C and Hart M. The worldwide environment of cardiovascular disease: prevalence, diagnosis, therapy, and policy issues: a report from the American College of Cardiology. *Journal of the American College of Cardiology*. 2012;60:S1-49.
2. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. *The New England journal of medicine*. 2013;368:2004-13.
3. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *The New England journal of medicine*. 2005;352:1685-95.
4. Galkina E and Ley K. Immune and inflammatory mechanisms of atherosclerosis (*). *Annual review of immunology*. 2009;27:165-97.
5. Libby P and Sasiela W. Plaque stabilization: Can we turn theory into evidence? *The American journal of cardiology*. 2006;98:26P-33P.
6. Finn AV, Nakano M, Narula J, Kolodgie FD and Virmani R. Concept of vulnerable/unstable plaque. *Arteriosclerosis, thrombosis, and vascular biology*. 2010;30:1282-92.
7. Moreno PR. The high-risk thin-cap fibroatheroma: a new kid on the block. *Circulation Cardiovascular interventions*. 2009;2:500-2.
8. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Judd SE, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Mackey RH, Magid DJ, Marcus GM, Marelli A, Matchar DB, McGuire DK, Mohler ER, 3rd, Moy CS, Mussolino ME, Neumar RW, Nichol G, Pandey DK, Paynter NP, Reeves MJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Wong ND, Woo D and Turner MB. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation*. 2014;129:e28-e292.
9. Monge M, Lorthioir A, Bobrie G and Azizi M. New drug therapies interfering with the renin-angiotensin-aldosterone system for resistant hypertension. *Journal of the renin-angiotensin-aldosterone system : JRAAS*. 2013;14:285-9.
10. Paulis L and Unger T. Novel therapeutic targets for hypertension. *Nature reviews Cardiology*. 2010;7:431-41.
11. Rajagopalan S, Bakris GL, Abraham WT, Pitt B and Brook RD. Complete renin-angiotensin-aldosterone system (RAAS) blockade in high-risk patients: recent insights from renin blockade studies. *Hypertension*. 2013;62:444-9.
12. Jensen C, Herold P and Brunner HR. Aliskiren: the first renin inhibitor for clinical treatment. *Nature reviews Drug discovery*. 2008;7:399-410.
13. Friedrich S and Schmieder RE. Review of direct renin inhibition by aliskiren. *Journal of the renin-angiotensin-aldosterone system : JRAAS*. 2013;14:193-6.
14. Rader DJ, Cohen J and Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *Journal of Clinical Investigation*. 2003;111:1795-1803.
15. National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002;106:3143-421.
16. Steinberg D. In celebration of the 100th anniversary of the lipid hypothesis of atherosclerosis. *Journal of lipid research*. 2013;54:2946-9.
17. Ridker PM. LDL cholesterol: controversies and future therapeutic directions. *Lancet*. 2014;384:607-17.
18. Tobert JA. Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. *Nature reviews Drug discovery*. 2003;2:517-26.
19. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J and Collins R. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170 000 participants in 26 randomised trials. *The Lancet*. 2010;376:1670-1681.

20. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R and Simes R. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. *The Lancet*. 2005;366:1267-1278.
21. Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, Barnes EH, Voysey M, Gray A, Collins R and Baigent C. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *The Lancet*. 2012;380:581-590.
22. Burnett JR and Huff MW. Cholesterol absorption inhibitors as a therapeutic option for hypercholesterolaemia. *Expert opinion on investigational drugs*. 2006;15:1337-51.
23. Princen HMG, Post SM and Twisk J. Regulation of bile acid biosynthesis. *Curr Pharm Des*. 1997;3:59-84.
24. Carlson LA. Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. *Journal of internal medicine*. 2005;258:94-114.
25. Kamanna VS, Ganji SH and Kashyap ML. Recent advances in niacin and lipid metabolism. *Current opinion in lipidology*. 2013;24:239-45.
26. Jandeleit-Dahm KAM, Calkin A, Tikellis C and Thomas M. Direct antiatherosclerotic effects of PPAR agonists. *Current opinion in lipidology*. 2009;20:24-29.
27. Lalloyer F and Staels B. Fibrates, glitazones, and peroxisome proliferator-activated receptors. *Arteriosclerosis, thrombosis, and vascular biology*. 2010;30:894-9.
28. Bijland S, Pieterman EJ, Maas AC, van der Hoorn JW, van Erk MJ, van Klinken JB, Havekes LM, van Dijk KW, Princen HM and Rensen PC. Fenofibrate increases very low density lipoprotein triglyceride production despite reducing plasma triglyceride levels in APOE*3-Leiden.CETP mice. *The Journal of biological chemistry*. 2010;285:25168-75.
29. Sakamoto J, Kimura H, Moriyama S, Odaka H, Momose Y, Sugiyama Y and Sawada H. Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone. *Biochemical and biophysical research communications*. 2000;278:704-11.
30. Horton JD, Cohen JC and Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *Journal of lipid research*. 2009;50 Suppl:S172-7.
31. Dubuc G, Chamberland A, Wassef H, Davignon J, Seidah NG, Bernier L and Prat A. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arteriosclerosis, thrombosis, and vascular biology*. 2004;24:1454-9.
32. Mayne J, Dewpura T, Raymond A, Cousins M, Chaplin A, Lahey KA, Lahaye SA, Mbikay M, Ooi TC and Chretien M. Plasma PCSK9 levels are significantly modified by statins and fibrates in humans. *Lipids in health and disease*. 2008;7:22.
33. Careskey HE, Davis RA, Alborn WE, Troutt JS, Cao G and Konrad RJ. Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. *Journal of lipid research*. 2008;49:394-8.
34. Furnsinn C, Willson TM and Brunmair B. Peroxisome proliferator-activated receptor-delta, a regulator of oxidative capacity, fuel switching and cholesterol transport. *Diabetologia*. 2007;50:8-17.
35. Ehrenborg E and Skogsberg J. Peroxisome proliferator-activated receptor delta and cardiovascular disease. *Atherosclerosis*. 2013;231:95-106.
36. Miller GJ and Miller NE. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet*. 1975;1:16-9.
37. Kingwell BA, Chapman MJ, Kontush A and Miller NE. HDL-targeted therapies: progress, failures and future. *Nature reviews Drug discovery*. 2014;13:445-64.
38. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A and Zukel WJ. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. *Circulation*. 1977;55:767-772.
39. Miller NE, Thelle DS, Forde OH and Mjos OD. The Tromso heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study. *Lancet*. 1977;1:965-8.

40. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG and Danesh J. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA : the journal of the American Medical Association*. 2009;302:1993-2000.
41. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR, Bangdiwala S and Tyroler HA. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation*. 1989;79:8-15.
42. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Holm H, Ding EL, Johnson T, Schunkert H, Samani NJ, Clarke R, Hopewell JC, Thompson JF, Li M, Thorleifsson G, Newton-Cheh C, Musunuru K, Pirruccello JP, Saleheen D, Chen L, Stewart A, Schillert A, Thorsteinsdottir U, Thorgeirsson G, Anand S, Engert JC, Morgan T, Spertus J, Stoll M, Berger K, Martinelli N, Girelli D, McKeown PP, Patterson CC, Epstein SE, Devaney J, Burnett MS, Mooser V, Ripatti S, Surakka I, Nieminen MS, Sinisalo J, Lokki ML, Perola M, Havulinna A, de Faire U, Gigante B, Ingelsson E, Zeller T, Wild P, de Bakker PI, Klungel OH, Maitland-van der Zee AH, Peters BJ, de Boer A, Grobbee DE, Kamphuisen PW, Deneer VH, Elbers CC, Onland-Moret NC, Hofker MH, Wijmenga C, Verschuren WM, Boer JM, van der Schouw YT, Rasheed A, Frossard P, Demissie S, Willer C, Do R, Ordovas JM, Abecasis GR, Boehnke M, Mohlke KL, Daly MJ, Guiducci C, Burtt NP, Surti A, Gonzalez E, Purcell S, Gabriel S, Marrugat J, Peden J, Erdmann J, Diemert P, Willenborg C, Konig IR, Fischer M, Hengstenberg C, Ziegler A, Buysschaert I, Lambrechts D, Van de Werf F, Fox KA, El Mokhtari NE, Rubin D, Schrezenmeir J, Schreiber S, Schafer A, Danesh J, Blankenberg S, Roberts R, McPherson R, Watkins H, Hall AS, Overvad K, Rimm E, Boerwinkle E, Tybjaerg-Hansen A, Cupples LA, Reilly MP, Melander O, Mannucci PM, Ardissino D, Siscovick D, Elosua R, Stefansson K, O'Donnell CJ, Salomaa V, Rader DJ, Peltonen L, Schwartz SM, Altshuler D and Kathiresan S. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet*. 2012;380:572-580.
43. Haase CL, Tybjaerg-Hansen A, Qayyum AA, Schou J, Nordestgaard BG and Frikke-Schmidt R. LCAT, HDL cholesterol and ischemic cardiovascular disease: a Mendelian randomization study of HDL cholesterol in 54,500 individuals. *The Journal of clinical endocrinology and metabolism*. 2012;97:E248-56.
44. Remaley AT, Norata GD and Catapano AL. Novel concepts in HDL pharmacology. *Cardiovascular research*. 2014;103:423-8.
45. van der Hoorn JW, de Haan W, Berbee JF, Havekes LM, Jukema JW, Rensen PC and Princen HM. Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester transfer protein in APOE*3Leiden.CETP mice. *Arteriosclerosis, thrombosis, and vascular biology*. 2008;28:2016-22.
46. Brown ML, Inazu A, Hesler CB, Agellon LB, Mann C, Whitlock ME, Marcel YL, Milne RW, Koizumi J, Mabuchi H and et al. Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. *Nature*. 1989;342:448-51.
47. Barter PJ and Rye KA. Cholesteryl ester transfer protein inhibition as a strategy to reduce cardiovascular risk. *Journal of lipid research*. 2012;53:1755-66.
48. van der Hoogt CC, de Haan W, Westerterp M, Hoekstra M, Dallinga-Thie GM, Romijn JA, Princen HM, Jukema JW, Havekes LM and Rensen PC. Fenofibrate increases HDL-cholesterol by reducing cholesteryl ester transfer protein expression. *Journal of lipid research*. 2007;48:1763-71.
49. Chapman MJ, Le Goff W, Guerin M and Kontush A. Cholesteryl ester transfer protein: at the heart of the action of lipid-modulating therapy with statins, fibrates, niacin, and cholesteryl ester transfer protein inhibitors. *European heart journal*. 2010;31:149-64.
50. Balder JW, Staels B and Kuivenhoven JA. Pharmacological interventions in human HDL metabolism. *Current opinion in lipidology*. 2013;24:500-9.
51. Franceschini G, Sirtori CR, Capurso A, 2nd, Weisgraber KH and Mahley RW. A-IMilano apoprotein. Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *The Journal of clinical investigation*. 1980;66:892-900.

52. Sirtori CR, Calabresi L, Franceschini G, Baldassarre D, Amato M, Johansson J, Salvetti M, Monteduro C, Zulli R, Muiesan ML and Agabiti-Rosei E. Cardiovascular status of carriers of the apolipoprotein A-I(Milano) mutant: the Limone sul Garda study. *Circulation*. 2001;103:1949-54.
53. de Knijff P, van den Maagdenberg AM, Stalenhoef AF, Leuven JA, Demacker PN, Kuyt LP, Frants RR and Havekes LM. Familial dysbetalipoproteinemia associated with apolipoprotein E3-Leiden in an extended multigeneration pedigree. *The Journal of clinical investigation*. 1991;88:643-55.
54. van den Maagdenberg AM, Hofker MH, Krimpenfort PJ, de Bruijn I, van Vlijmen B, van der Boom H, Havekes LM and Frants RR. Transgenic mice carrying the apolipoprotein E3-Leiden gene exhibit hyperlipoproteinemia. *The Journal of biological chemistry*. 1993;268:10540-5.
55. Westerterp M, van der Hoogt CC, de Haan W, Offerman EH, Dallinga-Thie GM, Jukema JW, Havekes LM and Rensen PC. Cholesteryl ester transfer protein decreases high-density lipoprotein and severely aggravates atherosclerosis in APOE*3-Leiden mice. *Arteriosclerosis, thrombosis, and vascular biology*. 2006;26:2552-9.
56. Zadelaar S, Kleemann R, Verschuren L, de Vries-Van der Weij J, van der Hoorn J, Princen HM and Kooistra T. Mouse models for atherosclerosis and pharmaceutical modifiers. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27:1706-21.
57. de Haan W, van der Hoogt CC, Westerterp M, Hoekstra M, Dallinga-Thie GM, Princen HM, Romijn JA, Jukema JW, Havekes LM and Rensen PC. Atorvastatin increases HDL cholesterol by reducing CETP expression in cholesterol-fed APOE*3-Leiden.CETP mice. *Atherosclerosis*. 2008;197:57-63.
58. de Haan W, de Vries-van der Weij J, van der Hoorn JW, Gautier T, van der Hoogt CC, Westerterp M, Romijn JA, Jukema JW, Havekes LM, Princen HM and Rensen PC. Torcetrapib does not reduce atherosclerosis beyond atorvastatin and induces more proinflammatory lesions than atorvastatin. *Circulation*. 2008;117:2515-22.