CHAPTER 7

Innovative Pharmaceutical Interventions in Cardiovascular Disease: Focusing on the Contribution of non-HDL-C/LDL-C-lowering versus HDL-C-raising

A systematic review and meta-analysis of relevant preclinical studies and clinical trials

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Abstract

Introduction Cholesterol contained in LDL particles is well recognized as a primary causal risk factor for cardiovascular disease. However, despite consistent epidemiological evidence for an inverse association between HDL-C and coronary heart disease, clinical trials aimed at raising HDL-C (AIM-HIGH, HPS2-THRIVE, dal-OUTCOMES) failed to meet their primary goals. This systematic review and meta-analysis investigated the effects of established and novel treatment strategies, specifically targeting HDL, on inhibition of atherosclerosis in cholesteryl ester transfer protein-expressing animals, and the prevention of clinical events in randomized controlled trials.

Methods and Results Linear regression analyses using data from preclinical studies revealed associations for TC and non-HDL-C and lesion area (R^2=0.258, P=0.045; R^2=0.760, P<0.001), but not for HDL-C (R^2=0.030, P=0.556). In clinical trials, non-fatal myocardial infarction risk was significantly less in the treatment group with pooled odd ratios of 0.87 [0.81; 0.94] for all trials and 0.85 [0.78; 0.93] after excluding some trials due to off-target adverse events, whereas all-cause mortality was not affected (OR 1.05 [0.99-1.10]). Meta-regression analyses revealed a trend towards an association between between-group differences in absolute change from baseline in LDL-C and non-fatal myocardial infarction (P=0.066), whereas no correlation was found for HDL-C (P=0.955).

Discussion We conclude that the protective role of lowering LDL-C and non-HDL-C is well-established. The contribution of raising HDL-C on inhibition of atherosclerosis and the prevention of cardiovascular disease remains undefined and may be dependent on the mode of action of HDL-C-modification. Nonetheless, treatment strategies aimed at improving HDL function and raising apolipoprotein A-I may be worth exploring.

Keywords HDL-C-raising pharmaceutical interventions; preclinical studies; randomized controlled trials; systematic review; meta-analysis

Content

1. Introduction

In 1913, Nikolai N. Anitschkow first described the involvement of cholesterol in atherosclerosis development when rabbits fed a high-cholesterol diet developed human-like arterial lesions.[1] The recent 100th year anniversary of this discovery is worth commemorating given that serum cholesterol contained in low-density lipoprotein (LDL) particles is now well recognized as a primary causal risk factor for cardiovascular disease as evidenced by experimental, epidemiological and genetic studies.[2] Indeed, intervention trials with statin therapy confirmed a reduced incidence of coronary heart disease as a consequence of cholesterol-lowering[3, 4] and recent trials indicated that intensive lipid-lowering with statins may be more beneficial in risk reduction than less intensive (or standard) therapy.[5] 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. However, treatment of cardiovascular disease remains suboptimal due to (i) the residual risk that persists after statin treatment,[6] (ii) failure for some patients to reach LDL-C targets despite statin treatment,[7] and (iii) lack of adherence as a result of statin intolerance.[8] Therefore, the search for secondary treatment targets is warranted.

In the 1970s, Miller & Miller hypothesized that a reduction in plasma high-density lipoprotein (HDL) concentration may accelerate the development of atherosclerosis and ischemic heart disease by impairing cholesterol clearance from the arterial wall.[9] Besides its major role in reverse cholesterol transport, HDL has also been described to have anti-inflammatory, anti-oxidant, anti-platelet and vasodilatory properties.[10] Although the original hypothesis referred to HDL particle concentration which could not be measured at the time,[10] epidemiological studies consistently reported an inverse association between coronary heart disease risk and HDL-C.[11-13] 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 coronary heart disease risk.[14]

Several therapeutic approaches aimed at raising HDL-C levels have since been investigated. However, undisputed proof for causality of low HDL-C in cardiovascular disease is lacking and clinical trials aimed at raising HDL-C to prevent disease (AIM-HIGH, HPS2-THRIVE, dal-OUTCOMES) have failed to meet their primary goals.[15-17] In addition, data from Mendelian randomization studies showed that genetic variants related to altered plasma HDL-C *per se* were not associated with risk of myocardial infarction,[18, 19] and that despite an inverse correlation, HDL-C and myocardial infarction risk are not causally related. Nonetheless, numerous therapeutic strategies aimed at raising HDL-C or improving HDL function are still under investigation in preclinical studies and clinical trials.

This systematic review investigated the effects of established and novel treatment strategies, specifically targeting HDL, on inhibition of atherosclerosis development in cholesteryl ester transfer protein (CETP)-expressing animals, since CETP is a crucial gene involved in HDL metabolism and implicated in the mechanisms by which most therapies modulate HDL.[20] 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, focusing specifically on the contribution of non-HDL-C/LDL-C-lowering versus HDL-C-raising.

2. Methods

The study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines using a PRISMA checklist.[21]

2.1 Preclinical studies

2.1.1 Literature search strategy

To identify relevant preclinical studies, we performed a computerized search of PUBMED and EMBASE. The search was restricted to studies published in the English-language from January 1975 to current. The following treatment strategies, specifically targeting HDL were included in the search: niacin; peroxisome proliferator-activated receptor (PPAR)-α agonists (fibrates); PPAR-γ agonist (glitazones); PPAR-α/γ agonists (glitazars); PPAR-δ agonists; CETP inhibitors; liver X receptor (LXR) agonists; micro RNAs; reconstituted HDL and apolipoprotein A-I-based compounds. We focused on preclinical studies evaluating the effects of these treatment strategies on atherosclerosis development in CETP-expressing animals. Statins were not included in the search criteria since the effects of statins on HDL/apolipoprotein A-I in relation to clinical outcomes were recently extensively reviewed.[22] Additional studies were identified by searching bibliographies from relevant studies and additional review articles.

2.1.2 Study selection

To investigate the role of HDL-C-raising treatment strategies on atherosclerosis development, we included preclinical studies that reported an increase in HDL-C. The effect of reconstituted HDL and apolipoprotein A-I-based compounds were described regardless of an effect on lipids, since several studies revealed protection against atherosclerosis with no change in plasma lipids. Studies were excluded if the relevant compound was used in a control group. All studies were screened to eliminate irrelevant studies by title and abstract. Remaining records were screened based on a review of the full text.

2.1.3 Quality assessment and data extraction

The following data were extracted from relevant preclinical studies: study design (compound, animal model, sex, diet, run-in phase, group size, dose and treatment phase), baseline and on-treatment serum/plasma total cholesterol (TC) and HDL-C levels, as well as atherosclerotic lesion area. The data were extracted by one author (SK) and thoroughly checked by another author (JWAvdH). Disagreements between authors were resolved by consensus.

2.1.4 Data presentation and analysis

To evaluate the effects of lipid-modifying treatment strategies on atherosclerosis, the percentage difference in atherosclerotic lesion area (gain) between the control and the treatment group was reported for all preclinical studies. Plasma/serum TC and HDL-C levels were retrieved for all time points reported in these studies. If not reported, non-HDL-C levels were calculated (TC – HDL-C). TC, non-HDL-C and HDL-C levels were standardized by converting mmol/L to mg/dL by multiplying by 38.67. Where possible, the percentage difference in TC, non-HDL-C and HDL-C exposure (duration of intervention in weeks x cholesterol levels) between the control and the treatment groups were calculated from the retrieved data and correlated with the between-group percentage difference in atherosclerotic lesion area. Reconstituted HDL and apolipoprotein A-I-based treatment strategies were not included in the correlations, but described in the discussion section due to different mechanisms of action.

2.1.5 Statistical analysis

Linear regression analyses were used to assess the association between the percentage difference in TC, non-HDL-C and HDL-C exposure and atherosclerotic lesion area between the control and the treatment groups.

2.2 Randomized controlled clinical trials

2.2.1 Literature search strategy

To find relevant randomized controlled clinical trials, we performed a search of PUBMED including studies from clinicaltrials.gov, a clinical trial registry and results database, and EMBASE. We included the same restrictions and treatment strategies as previously described for preclinical studies (see 2.1.1). The search involved clinical trials reporting major cardiovascular events and we searched for phase II, III and IV clinical trials, multicenter, randomized controlled trials and meta-analyses. Additional studies were identified by searching bibliographies from relevant trials, as well as meta-analyses and review articles. The study authors were not contacted regarding the retrieval of unpublished data.

2.2.2 Study selection

Inclusion criteria used for the selection of clinical trials for pooled meta-analyses were as follows:

- A randomized placebo-controlled trial design
- Patients with type 2 diabetes or cardiovascular disease or patients at risk of developing cardiovascular disease
- A trial sample size of ≥ 200 participants in each study arm
- A mean follow up duration of ≥ 1 year
- Pharmaceutical HDL-C-raising agents
- At least two of the following clinical outcomes: all-cause mortality, coronary heart disease mortality, non-fatal myocardial infarction and stroke

After discarding irrelevant records based on title and abstract, relevant articles were selected based on full text screening.

2.2.3 Quality assessment and data extraction

Study design (compound, study population, follow up duration and sample size), baseline characteristics (age, sex, BMI, history of cardiovascular disease, diabetes, hypertension, smoking, myocardial infarction, stroke, angina, revascularization, heart failure, peripheral vascular disease and previous statin use), baseline and on-treatment TC, HDL-C, LDL-C and non-HDL-C levels, as well as the occurrence of clinical events (all-cause mortality, coronary heart disease mortality, non-fatal myocardial infarction and stroke) were obtained from selected clinical trials. The data were extracted by one author (SK) and thoroughly checked by another author (JWAvdH). Disagreements between authors were resolved by consensus.

2.2.4 Data presentation and analysis

We performed 4 separate meta-analyses to analyze the effects of treatment on the prevention of all-cause mortality, coronary heart disease mortality, non-fatal myocardial infarction and stroke in randomized controlled trials. These 4 meta-analyses were repeated after excluding a number of trials using compounds with serious off-target cardiovascular adverse events. These include trials with torcetrapib and aleglitazar of which clinical development was stopped due to adverse effects, as well as pioglitazone which was shown to increase heart failure.[23-28] Other primary endpoint data were not included in this meta-analysis, because of different composite endpoints for the various trials. In addition, we performed 2 meta-analyses to assess the effects of treatment on the prevention of non-fatal myocardial infarction in patients with low versus high baseline LDL-C by dividing the remaining trials into 2 subgroups using LDL-C levels of 100 mg/dL as the cut-off. In fact, the trials with lower baseline LDL-C levels concern patients on statin treatment (60%-100% of the subjects). In this regard, patients in the ACCORD trial had an average LDL-C of 100.6

mg/dL, and 60% of these patients received statin treatment at trial entry. We, therefore, included this study in the subgroup analysis of patients with low baseline LDL-C.[29]

Baseline and on-treatment TC, LDL-C, non-HDL-C and HDL-C levels were standardized by converting mmol/L to mg/dL by multiplying by 38.67. When lipid data were presented for multiple time points, we reported results from the longest follow up period. To determine the between-group differences in lipid changes, we calculated the difference between the absolute and the percentage change from baseline in the control group and the treatment group. Meta-regression analyses were performed to assess the potential association between the between-group differences in absolute and percentage change from baseline in LDL-C, as well as HDL-C and the occurrence of non-fatal myocardial infarction.

2.2.5 Statistical analysis

A random effects model was employed to pool clinical trial-specific odds ratios in order to estimate an overall odds ratio and its associated confidence intervals. Inverse variance method which gives more weight to larger trials was used to pool outcomes for different trials. The overall effects corresponding to a fixed and random effects model are reported together in the same forest plot along with their confidence intervals. The sizes of the square boxes on the forest plots are proportional to the total number of patients in the selected trials. An overall test on heterogeneity between studies was performed for each separate meta-analysis (value I-squared in figures). To estimate the between-study variance, which is represented as 'tau' in the forest plots, DerSimonian-Laird's method has been employed.[30] The log-transformed odd ratios for myocardial infarction was modeled as a linear function of the between-group differences in absolute and percentage change from baseline in LDL-C, as well as HDL-C by employing meta-regression. All statistical analyses were performed using R version 2.18. (http://cran.rproject.org/).

3. Results

3.1 Preclinical studies

3.1.1 Reference screening

The computerized search identified 967 records of which 119 duplicates were removed. The remaining 848 records were screened based on title and abstract and an additional 729 records were excluded. After reviewing 119 full text articles, 92 irrelevant records were removed and the results of 29 preclinical studies, including 2 studies that were not identified in the original search, were included in the systematic review (**Figure 1**).

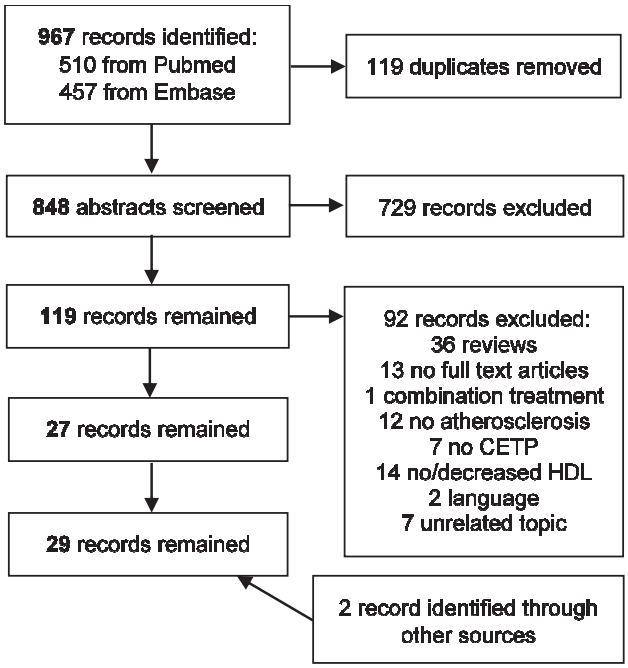

Figure 1 An outline of the systematic search conducted to identify relevant preclinical studies for the systematic review.

3.1.2 Study design and baseline lipid levels

The study design and baseline lipid levels for the selected preclinical studies are summarized in **Table 1**. In these studies, the effects of niacin,[31, 32] PPAR-α agonists,[33, 34] PPAR α/γ agonist,[35] CETP antisense,[36] CETP vaccines,[37-40] CETP inhibitors,[41-45] SR-BI inhibitor,[46] ABCA1 degradation inhibitors,[47] purified or reconstituted HDL,[48-51] apolipoprotein A-I Milano[52-57] and apolipoprotein A-I mimetic peptide[58, 59] on atherosclerosis development were investigated in APOE*3Leiden.CETP and *ldlr*$^{+/-}$.CETP mice, New Zealand White (NZW), Japanese White (JW) and Watanabe heritable hyperlipidemic (WHHL) rabbits, as well as F1B hamsters. Whereas several studies were designed to detect a reduction in the progression of atherosclerosis, a number of lipid-modifying treatment studies[33-35] and most purified and reconstituted HDL and apolipoprotein A-I-based studies investigated the effects of treatment on existing atherosclerosis in a regression set-up. All animals received a cholesterol-containing diet except for WHHL rabbits, which spontaneously develop atherosclerosis. In a number of studies, atherosclerosis was induced by collar placement, electric and balloon injury/denudation.[33, 51-57] The effects of PPAR-δ agonists[60] and miR-33 antagonism[61] on atherosclerosis development were investigated in animals that do not express CETP and according to or knowledge have not been tested in an animal model with a more human-like

lipoprotein metabolism. Studies of LXR and FXR agonists in CETP-expressing animals were not included in this review, since no increase in HDL-C was observed except for the study by Srivastava *et al.* evaluating the anti-atherosclerotic activities of PPAR-α, PPAR-γ and LXR agonist (T0901317) in F1B hamsters.[34]

3.1.3 Effect of lipid-modifying treatment strategies on atherosclerosis development

All lipid-modifying treatment strategies decreased atherosclerotic lesion area, except dalcetrapib.[42] In this study, the treatment also failed to reduce TC levels despite an increase in HDL-C.

Table 1 Design and baseline lipid levels of the selected preclinical studies.

Study	**Year**	**Compound**	**Animal model**	**Sex**	**Diet**	**Run-in phase** (weeks)	**Group size** C/T	**Dose** (mg/kg/d)	**Treatment phase** (weeks)	**Baseline TC/*LDL-C*** (mg/dL)	**Baseline HDL-C** (mg/dL)
Parwaresch	1978	Nicotinic acid Xantinol-nicotinate* β-pyridylcarbinol* Pirozadil*	NZW rabbits	M	Diet + 3% cholesterol	0	26/19 26/33 26/28 26/19	50 50 50 50	12	958.0	19.0
Kühnast	2013	Niacin	E3L.CETP mice	F	WTD + 0.1% cholesterol	3	15/15	120	18	440.8	21.3
Corti	2007	Fenofibrate	NZW rabbits	M	Diet + 0.2% cholesterol	39: Induction after 4 & 13	6/6	ns	26	*736.7*	57.3
van der Hoorn	2009	Tesaglitazar	E3L.CETP mice	F	WTD + 0.3% or 0.1% cholesterol	15: 11 high + 4 low cholesterol	16/16	10 µg	8: low cholesterol	421.5	ns
Srivastava	2011	Fenofibrate	F1B hamsters	ns	High fat high cholesterol	2 11	11/11 10/10	100 100	13	ns	ns
Sugano	1998	CETP antisense oligonucleotides	JW rabbits	M	Chow + 0.3% cholesterol	8	6/6	30 µg/kg 2x/week	8	270.0	19.0
Rittershaus	2000	CETP vaccine	NZW rabbits	ns	Chow + 0.25% cholesterol	0	12/12	Week 1, 5, 8, 16, 22	32: high cholesterol from 16	ns	ns
Gaofu	2005	CETP vaccine	NZW rabbits	F	Chow + 0.25% cholesterol	0	6/6	Week 1, 5, 10, 16, 22	26: high cholesterol from 12	130.7	7.0
Mao	2006	CETP vaccine	NZW rabbits	F	Diet + 0.5g cholesterol	0	5/5	Week 1, 3, 7, 11, 16, 22	26: high cholesterol from 11	130.7	7.0
Jun	2012	CETP vaccine	NZW rabbits	M	Diet + 0.5% cholesterol	0	8/8	Week 0, 2, 4, 6, 8	25: high cholesterol from 9	ns	ns
Okamoto	2000	Dalcetrapib	JW rabbits	M	Chow + 0.2% cholesterol	5	10/10	255	26	274.0	17.5
Huang	2002	Dalcetrapib	JW rabbits	M	Chow + 0.25% cholesterol	4	10/8 10/10	100 300	12	406.0	21.0
Morehouse	2007	Torcetrapib	NZW rabbits	M	Diet + 0.2% cholesterol	5 days	23/24	90 to 60	16: 3 dose finding	122.0	57.0 (after 1 week)
De Haan	2008	Torcetrapib	E3L.CETP mice	F	WTD + 0.25% cholesterol	4	15/15	12	14	653.5	ns
Kühnast	2014	Anacetrapib	E3L.CETP mice	F	WTD + 0.1% cholesterol	5	15/15 15/15 15/15 15/15	0.03 0.3 3 30	21	514.3	41.4
Masson	1990	SRBI inhibitor, ITX5061	*ldlr*$^{+/-}$ mice AAV CETP	ns	Diet + 1.25% cholesterol	0	10/10	0.037%	18	509.0	54.1
Arakawa	2009	ABCA1 degradation inhibitors, spiroquinone and diphenoquinone	NZW rabbits	M	Diet + 0.5% cholesterol	0	8/8 8/8	25 25	8	ns	19.0

Badimon	1990	HDL-VHDL plasma fraction	NZW rabbits	ns	Chow + 0.5% cholesterol	9	7/7	50 mg HDL/VHDL protein (1x/week)	4	1559.0	ns
Mezdour	1995	Reconstituted apoAI-containing HDL ± reconstituted TG-rich lipoprotein	JW rabbits	M	Diet + 0.5% cholesterol	0	7/6 7/4	18 mg apoAI-rHDL + Intralipid 18 mg apoAI-rHDL + 2.5 ml rTRL + 4 mg apoE (1x/week)	8	58.0	25.0
Miyazaki	1995	Purified rabbit apoAI	NZW rabbits	M	Chow ± 0.5% cholesterol	9:w cholesterol 15:w cholesterol	14/14 8/8 8/8	40 mg apoAI/week 1 mg apoAI/2 days 40 mg apoAI/week	4: w cholesterol 9: w/o cholesterol 9: w/o cholesterol	2500.0 2000.0 2000.0	ns ns ns
Nicholls	2005	Reconstituted apoAI-containing HDL or rabbit HDL	NZW rabbits	M	Chow + 0.2% cholesterol	16: Induction after 1	15/6 15/5 15/8	2x 25 mg apoAI + PLPC 2x 25 mg apoAI + DPPC Rabbit HDL	5 days (treatment on day 1 and 3)	483.4	ns
Ameli	1994	Recombinant apoAI Milano	NZW rabbits	M	Diet + 1% cholesterol	18 days	4/8	40 mg $apoAI_M$ + PC-C (/2 days for 10 days)	4: Induction 4 days after start of treatment	606.0	24.0
Soma	1995	Recombinant apoAI Milano dimer	NZW rabbits	M	Diet + 1% cholesterol	3: Induction after 3 weeks	5/5 5/5	40 mg $apoAI_M$ + EPC (at -5, -3, -1, 1, 3 days) 40 mg $apoAI_M$ + EPC (at 0, 2, 4, 6, 8 days)	10 days	930.0	ns
Chiesa	2002	Recombinant apoAI Milano	NZW rabbits	M	Diet + 1.5% cholesterol	13: Induction at start	5/5 5/5 5/5	250 mg $apoAI_M$ + DPPC 500 mg $apoAI_M$ + DPPC 1000 mg $apoAI_M$ +DPPC	3 days	ns	ns
Parolini	2008	Recombinant apoAI Milano (ETC-216)	NZW rabbits	M	Diet + 1.5% cholesterol	13: Induction at start	5/6 5/4 5/6 5/5 5/5	5 mg/kg $apoAI_M$ 10 mg/kg $apoAI_M$ 20 mg/kg $apoAI_M$ 40 mg/kg $apoAI_M$ 150 mg/kg $apoAI_M$ (5x every 4 days)	4 (until 1 week after last infusion)	ns	ns
Ibanez	2008	Recombinant apoAI Milano (ETC-216)	NZW rabbits	M	Diet + 0.2% cholesterol	39: Induction after 1 & 12	18/22	75 mg/kg $apoAI_M$ (2x every 4 days)	1 (until 4 days after last infusion)	ns	ns
Ibanez	2012	Recombinant apoAI Milano (ETC-216) or apo A-I wild type	NZW rabbits	M	Diet + 0.2% cholesterol	39: Induction after 1 & 12	5/5 5/5	75 mg/kg $apoAI_M$ 75 mg/kg $apoAI_{WT}$ (2x every 4 days)	1 (until 4 days after last infusion)	ns	ns
van Lenten	2007	ApoAI mimetic peptide (D-4F/L-4F)	NZW rabbits	F	Diet + 1% cholesterol	4	15/15 15/15	10 mg/kg/d D-4F 10 mg/kg/d L-4F	4	1963.0	ns
Iwata	2011	ApoAI mimetic peptide (ETC-642)	WHHL-MI rabbits	ns	Chow	0	8/5 8/8	15 mg/kg $apoAI_{MP}$ 50 mg/kg $apoAI_{MP}$ (2x/week)	12	1098.0	12.0

*nicotinic acid derivatives; AA- balloon inj, Abdominal Aorta balloon denudation injury; apo A-I, apolipoprotein A-I; apo $A\text{-}I_M$, apolipoprotein A-I Milano; apo $A\text{-}I_{MP}$, apolipoprotein mimetic peptide; apo $A\text{-}I_{WT}$, apolipoprotein A-I wild type; CA- collar, carotid artery - non-occlusive collar placement; CA- electric inj, Carotid Artery – electric perivascular injury; CETP, cholesteryl ester transfer protein; chol, cholesterol; DPPC, 1,2-dipalmitoyl phosphatidylcholine; E3L.CETP, apoE*3Leiden.CETP; EPC, egg phosphatidylcholine; F, female; HDL-VHDL, high-density lipoprotein-very high-denstiy lipoprotein; JW, Japanese White; *Idlr*$^{+/-}$AAV CETP, low desity lipoprotein receptor heterozygous mice injected with adeno-associated virus CETP; M, male; ns, not specified; NZW, New Zealand White; PC-C, phosphatidylcholine-choline; PLPC, 1-palmitoyl-2-linoleoyl phosphatidylcholine; rHDL, reconstituted HDL; rTRL, reconstituted triglyceride-rich lipoprotein; SRBI, scavenger receptor B-I; TG, triglyceride; WHHL-MI, Watanabe-heritable hyperlipidemic myocardial infarction; WTD, western-type diet

3.1.4 The association between TC/non-HDL-C/HDL-C and atherosclerotic lesion area

To further explore the atheroprotective role of non-HDL-C and HDL-C, a linear regression model was employed to study the association between the between-group percentage difference in TC, non-HDL-C and HDL-C exposure and atherosclerotic lesion area (**Figure 2**). Whereas TC and non-HDL-C associated with lesion area (R^2=0.258, P=0.045 and R^2=0.760, P<0.001, respectively), no correlation was found for HDL-C (R^2=0.030, P=0.556). After excluding an extreme data point (400% increase in HDL-C), both TC and non-HDL-C strongly correlated with lesion area (R^2=0.695, P<0.001 and R^2=0.818, P<0.001, respectively), but the association was still much less apparent for HDL-C (R^2=0.155, P=0.183).

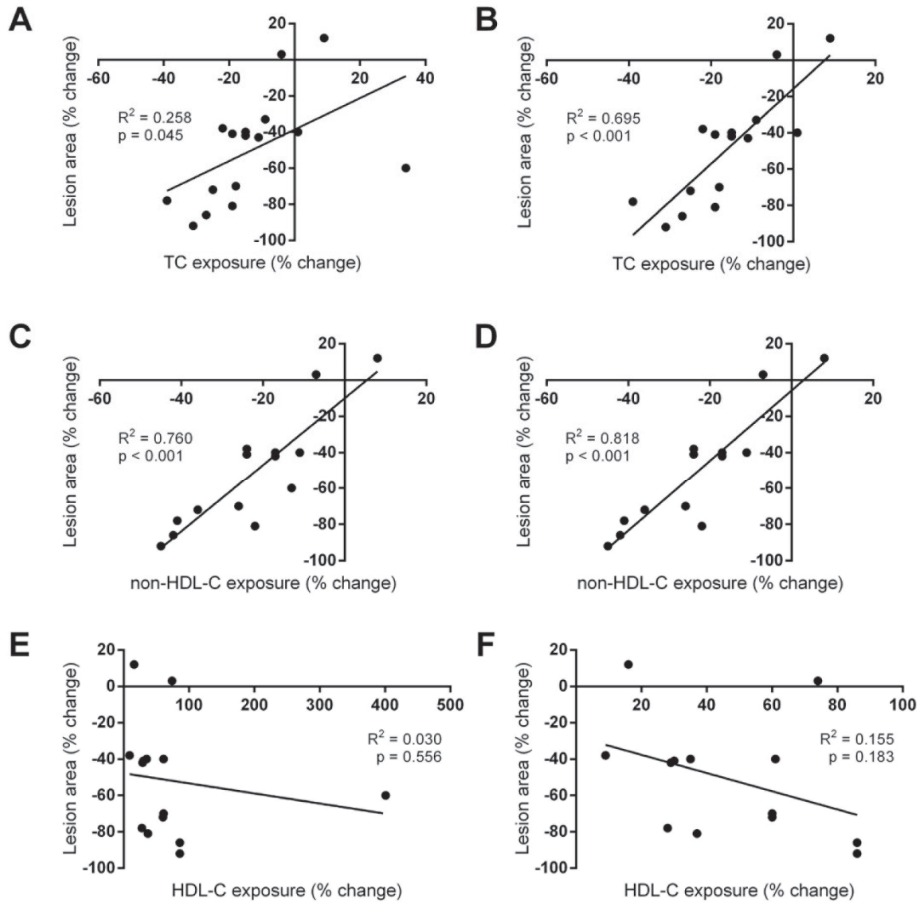

Figure 2 The correlation between percentage differences in plasma TC (A and B), non-HDL-C (C and D), as well as HDL-C (E and F) exposure and atherosclerotic lesion area between the control and treatment groups for all preclinical studies (A, C, E) and after excluding an extreme data point (400% increase in HDL-C) (B, D, F), respectively.

3.2 Randomized controlled clinical trials

3.2.1 Reference screening

The titles and abstracts of 629 records excluding 147 duplicates that were identified in the computerized search were reviewed and 287 records were removed. After retrieving 195 full text articles, 181 records were removed due to failure to meet the inclusion criteria. Together with an additional 8 articles that were identified from the bibliographies of relevant trials, meta-analysis and review articles, a total number of 22 randomized controlled trials were included in the meta-analysis (**Figure 3**).

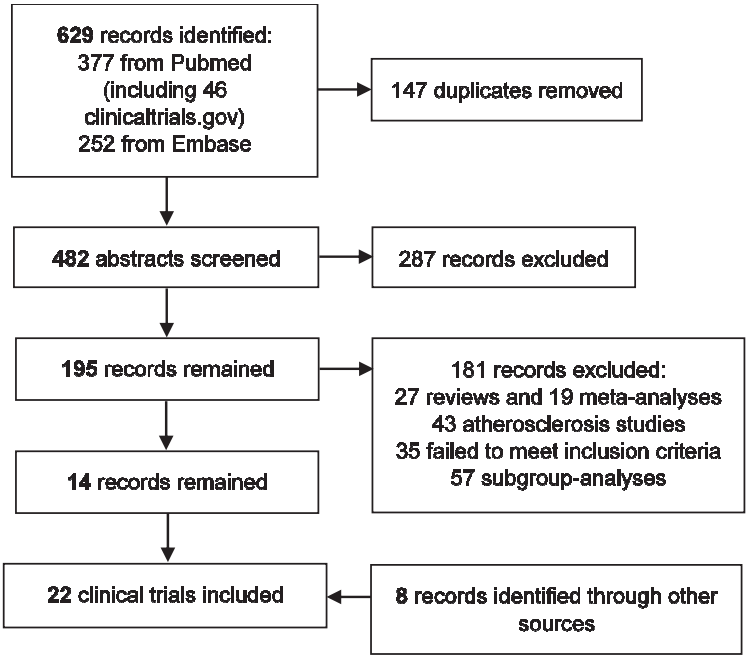

Figure 3 An outline of the systematic search conducted to identify relevant randomized controlled clinical trials for the meta-analysis.

3.2.2 Trial design and baseline characteristics

The trial design and baseline characteristics for the 22 randomized controlled trials that met the inclusion criteria are reviewed in **Table 2**. These trials evaluated the effects of PPAR-α agonists,[29, 62-72] niacin,[15, 16, 72] CETP inhibitors,[17, 23-26, 73] as well as PPAR-γ[27] and PPAR-α/γ agonists[28] on clinical outcomes. The 22 trials enrolled a total number of 121 666 patients: 61 093 in the control group and 60 573 in the treatment group. The mean duration of follow up was 3.8 years. The CDP trial investigated the effects of both clofibrate and niacin in two separate groups and we described this trial as two separate entities.[72]

Table 2 Design and baseline characteristics of the selected randomized controlled clinical trials.

Trial	**Year**	**Compound**	**Study population**	**Follow up** (yrs)	**Sample size** Total (Control/ Treatment)	**Age** (yrs)	**Men** (%)	**CVD** (%)	**Diabetes** (%)	**Previous statin use** (%)	**Baseline LDL-C** (mg/dL)	**Baseline HDL-C** (mg/dL)
CDP (clofibrate)	1975	Clofibrate	CHD	6.2	3892 2789/1103	ns	100	100	40	none	ns	ns
WHO	1978	Clofibrate	Healthy men	5.3	10627 5296/5331	46	100	0	0	none	ns	ns
DIS	1991	Clofibric acid	NIDDM	5.0	761 382/379	46	56	ns	100	ns	ns	ns
HHS	1987	Gemfibrozil	Dyslipidemia	5.0	4081 2030/2051	47	100	0	3	none	188.7	47.4
HHS Frick	1993	Gemfibrozil	Dyslipidemia with CHD symptoms	5.0	628 317/311	49	100	ns	ns	ns	188.3	46.4
VA-HIT	1999	Gemfibrozil	CHD	5.1	2531 1267/1264	64	100	100	25	ns	111.5	32.0
BIP	2000	Bezafibrate	CAD	6.2	3090 1542/1548	60	91	100	10	ns	148.5	34.6
LEADER	2002	Bezafibrate	Lower extremity arterial disease	4.6	1568 785/783	68	100	100	17	ns	131.5	46.0
DAIS	2001	Fenofibrate	T2D ± CHD	3.3	418 211/207	57	73	48	100	ns	131.7	39.8
FIELD	2005	Fenofibrate	T2D ± CVD	5.0	9795 4900/4895	62	63	22	100	0	118.7	42.5
ACCORD	2010	Fenofibrate	T2D + CVD or high risk for CVD	4.7	5518 2753/2765	62	69	37	100	60	100.6	38.1
FIRST	2014	Fenofibric acid	Dyslipidemia	2.0	682 342/340	61	68	22	50	63	84.3	39.9
CDP (niacin)	1975	Niacin	CHD	6.2	3908 2789/1119	ns	100	100	39	none	ns	ns
AIM-HIGH	2011	Niacin	CVD	3.0	3414 1696/1718	64	85	100	34	94	74.1	34.7

HPS2-THRIVE	2014	Niacin	CVD	3.9	25673 12835/12838	65	83	100	32	76	63.5	44.0
RADIANCE 1	2007	Torcetrapib	FH	2.0	904 454/450	46	49	ns	3	ns	138.7	52.4
RADIANCE 2	2007	Torcetrapib	Dyslipidemia	1.8	752 375/377	57	64	ns	21	ns	100.5	47.6
ILLUMINATE	2007	Torcetrapib	CVD or high risk for CVD or T2D	1.5	15067 7534/7533	61	78	ns	44	ns	79.8	48.6
ILLUSTRATE	2007	Torcetrapib	CHD	2.0	1188 597/591	57	70	100	21	91	83.7	45.6
DEFINE	2010	Anacetrapib	CHD or high risk for CHD	1.5	1623 812/811	63	77	55	53	99	81.8	40.5
dal-OUTCOMES	2012	Dalcetrapib	CHD + recent ACS	2.6	15871 7933/7938	60	81	100	24	97	76.1	42.4
PROactive	2005	Pioglitazone	T2D + macro vascular disease	2.9	5238 2633/2605	62	66	100	100	43	ns	ns
AleCardio	2014	Aleglitazar	T2D + recent ACS	2.0	7226 3610/3616	61	73	100	100	93	79.5	42.0

ACS, acute coronary syndrome; CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; FH, familial hypercholesterolemia; NIDDM, non-insulin dependent diabetes mellitus; ns, not specified; T2D, type 2 diabetes

3.2.3 Effect of lipid-modifying treatment strategies on clinical outcomes

All 22 trials were included in the analysis of all-cause mortality. Trials that did not report coronary heart disease mortality, non-fatal myocardial infarction and stroke were excluded from the analyses. The occurrence of all-cause mortality tended to be more frequent after lipid-modifying treatment as compared to the control with pooled odds ratios obtained by employing a random effects model of 1.05 [0.99; 1.10] for all studies and 1.04 [0.99; 1.10] after excluding a number of trials due to serious off-target adverse events (data not shown). The risk of non-fatal myocardial infarction was significantly less in the treatment group for an analysis of all trials (**Figure 4**) and after excluding a number of trials due to off-target adverse events (data not shown). A significant heterogeneity was observed for non-fatal myocardial infarction (I^2=40.8%, P=0.025). However, the pooled odds ratio obtained by employing a random effects model was 0.87 [0.81; 0.94] for all trials and 0.85 [0.78; 0.93] after excluding trials with off-target effects. No significant differences were observed in the occurrence of coronary heart disease mortality or stroke between the control and treatment groups (data not shown).

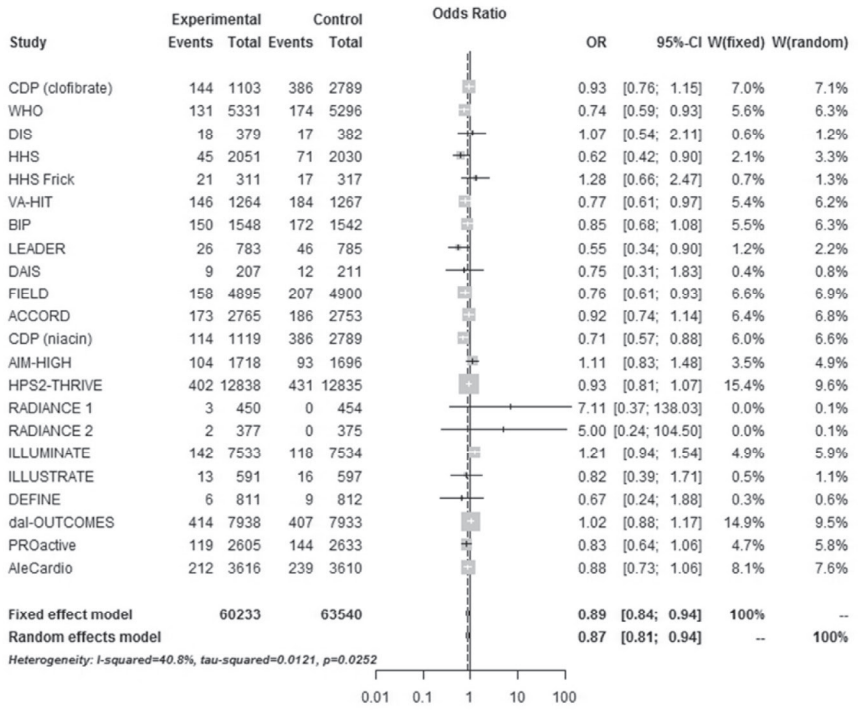

Figure 4 Forest plot for the effects of lipid-modifying treatment strategies, specifically targeting HDL-C, other than statins (PPAR-α agonists, niacin, CETP inhibitors, PPAR-γ and PPAR-α/γ agonists) on the occurrence of non-fatal myocardial infarction for all trials, demonstrating a significant risk reduction for treated subjects.

3.2.4 Effect of treatment on the prevention of non-fatal myocardial infarction in patient populations with low versus high baseline LDL-C

To assess the effects of lipid-modifying treatment on the prevention of non-fatal myocardial infarction in patients with high baseline LDL-C[62, 63, 65-69] versus low baseline LDL-C,[15-17, 29, 73] we performed 2 separate meta-analyses by dividing the remaining trials into 2 subgroups using LDL-C levels of 100 mg/dL as the cut-off. In patients with high baseline LDL-C, the occurrence of non-fatal myocardial infarction was significantly lower after lipid-modifying treatment as compared to patients in the control group (0.77 [0.68; 0.86]; **Figure 5A**). However, lipid-modifying treatment strategies failed to prevent the occurrence of non-fatal myocardial infarction in patients with low baseline LDL-C (0.97 [0.89; 1.06]; **Figure 5B**).

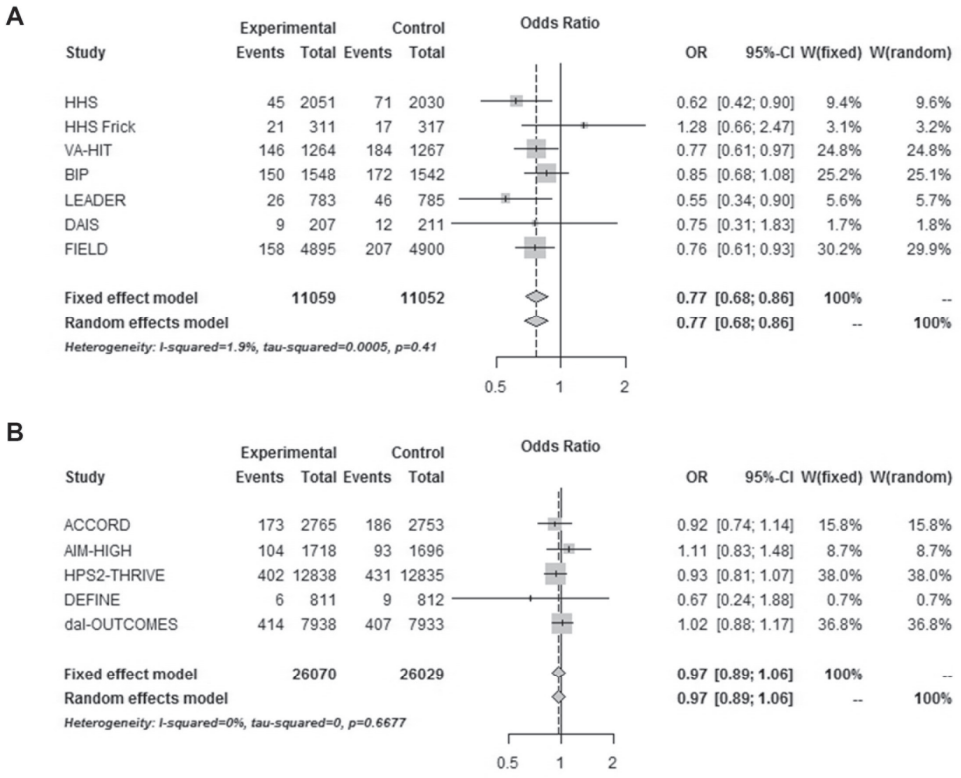

Figure 5 Forest plots for the effects of lipid-modifying treatment strategies, specifically targeting HDL-C, other than statins (PPAR-α agonists, niacin and CETP inhibitors) on the occurrence of non-fatal myocardial infarction in patient populations with baseline LDL-C > 100 mg/dL (A) and baseline LDL-C < 100 mg/dL (B), only revealing a significant risk reduction for treated subjects with baseline LDL-C > 100 mg/dL.

3.2.5 The association between LDL-C/HDL-C and non-fatal myocardial infarction

Meta-regression analyses revealed a trend towards an association between between-group differences in absolute change from baseline in LDL-C and non-fatal myocardial infarction (P=0.066; **Figure 6A**), whereas no correlation was found for HDL-C (P=0.955; **Figure 6B**).

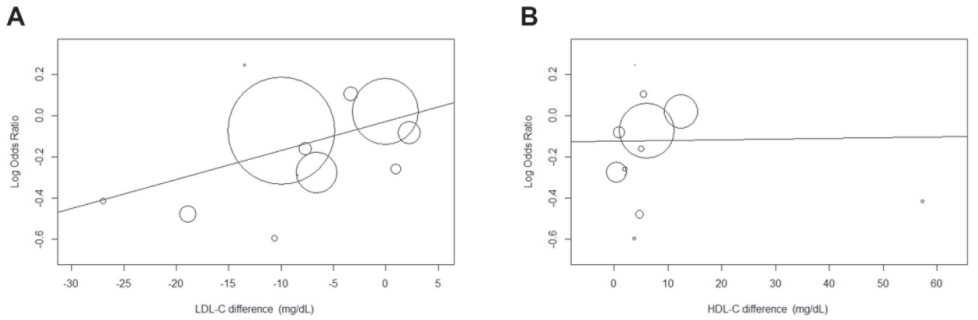

Figure 6 The association between absolute between-group differences in LDL-C and HDL-C change from baseline (mg/dL) and non-fatal myocardial infarction (log[OR]) in randomized controlled trials involving lipid-modifying treatment strategies, specifically targeting HDL-C, other than statins (PPAR-α agonists, niacin and CETP inhibitors), demonstrating a trend toward a positive correlation for LDL-C (P=0.066) and no correlation for HDL-C (P=0.955).

4. Discussion

The current systematic review and meta-analysis confirm the importance of cholesterol-lowering in the treatment of atherosclerosis and cardiovascular disease. However, based on data from preclinical studies and clinical trials, the protective effect of raising HDL-C is less defined and may be dependent on the mode of action of HDL-C-modification.

4.1 Treatment strategies aimed at raising HDL-C and atherosclerosis: preclinical studies

This systematic review investigated the effects of established and novel treatment strategies other than statins, specifically targeting HDL, on inhibition of atherosclerosis in animals expressing CETP. Most rodent models for atherosclerosis, for example *apoe*$^{-/-}$ and *ldlr*$^{-/-}$ mice, lack this crucial gene involved in HDL metabolism.[74] APOE*3Leiden.CETP mice express human CETP under control of its natural flanking regions.[75] These mice have impaired clearance of apolipoprotein B-containing lipoproteins and mimic the slow clearance observed in humans, particularly in patients with familial dysbetalipoproteinemia.[76] The APOE*3Leiden.CETP mice develop diet-induced atherosclerosis and respond to lipid-lowering and HDL-C-raising drugs in a human-like manner.[32, 44, 45, 77-82] Rabbits and hamsters naturally express CETP and develop diet-induced atherosclerosis. Nonetheless, it should be noted that cholesterol-fed rabbits develop lesions that predominantly consist of foam cells and do not represent

advanced lesions as observed in humans.[58] WHHL rabbits, a LDL receptor-deficient model for familial hypercholesterolemia spontaneously develop severe atherosclerotic lesions that are morphologically more similar to human lesions.[83] Hamsters are not a widely used as a model for atherosclerosis since it takes considerable time to develop atherosclerosis, and the lesions have a spotty-like appearance covering the whole aorta.

Using data from relevant animal studies that studied the effects of lipid-modifying treatment on atherosclerosis development as described below, we found significant correlations between both TC and non-HDL-C exposure and atherosclerosis (between-group percentage difference), however, there was no significant association between HDL-C and atherosclerosis.

4.1.1 Niacin

The benefits of niacin on plasma lipids was first described in 1955 and led to the development of niacin for therapeutic purposes.[84] Several mechanisms have been proposed for the beneficial effects of niacin on lipid metabolism. These include decreased free fatty acid flux from adipose tissue to the liver, decreased TG synthesis, increased apolipoprotein A-I lipidation and decreased apolipoprotein A-I removal, as well as inhibition of CETP.[20, 80, 85] In addition, niacin also exerts anti-inflammatory and anti-oxidant effects independent of lipid-lowering.[85]

In NZW rabbits, the increase in HDL-C/LDL-C ratio after treatment with different nicotinic acid derivatives resulted in a reduction in aortic surface area.[31] In APOE*3Leiden.CETP mice, the reduction in atherosclerotic lesion area was mostly accounted for by a decrease in non-HDL-C, although to some extent HDL-C predicted lesion area independent of non-HDL-C.[32] In the latter study, the combination of niacin and simvastatin reduced non-HDL-C beyond the level reached by simvastatin monotreatment and largely explained why niacin added to the anti-atherosclerotic effects of simvastatin.

4.1.2 Peroxisome proliferator-activated receptor (PPAR) agonists

PPARs are transcription factors involved in regulation of target gene expression. The effects of PPAR isoforms (α, γ) on glucose and lipid metabolism have led to the development of PPAR agonists for the treatment of hyperglycemia and dyslipidemia.[86, 87]

4.1.2.1 PPAR-α agonists

PPAR-α activation by fibrates increases lipoprotein lipase-mediated lipolysis, VLDL remnants clearance, β-oxidation,[88] apolipoprotein A-I/II expression and cholesterol efflux from macrophages.[86, 87] In addition, the HDL-C-raising effects of fibrates were ascribed to a reduction in CETP.[20, 79] Fibrates also inflict direct anti-oxidant and anti-inflammatory effects.[86, 89]

In NZW rabbits and F1B hamsters, fenofibrate reduced atherosclerosis progression and induced regression.[33, 34] In the rabbit model, the beneficial effects of fenofibrate were ascribed to an increase in HDL-C or potential pleiotropic effects of fibrates, since no significant change in LDL-C was observed.

4.1.2.2 PPAR-γ agonists

PPAR-γ agonists (glitazones) mainly mediate glucose homeostasis,[86] but pioglitazone also weakly activates PPAR-α which explains the small increase in HDL-C.[90] Similar to fibrates, glitazones exert anti-inflammatory and anti-oxidant effects independent of its metabolic activities. The atheroprotective effects of glitazones in preclinical studies were mostly independent of lipid modulation and according to our knowledge no studies reported an increase in HDL-C.

4.1.2.3 PPAR-α/γ agonists

PPAR-α/γ agonists (glitazars) were developed to more effectively improve lipid and glucose metabolism.[86] In APOE*3Leiden.CETP mice, tesaglitazar prevented progression of pre-existing atherosclerosis.[35] A strong reduction in non-HDL-C was observed, as well as an increase in HDL-C. The latter was not accompanied by a rise in apolipoprotein A-I, suggesting an increase in particle size rather than the number of particles.

4.1.3 Cholesteryl ester transfer protein (CETP) inhibition

In 1989, markedly increased HDL-C led to the discovery of the first mutation in the CETP gene in two Japanese subjects.[91] CETP facilitates the transfer of cholesteryl esters from atheroprotective HDL to atherogenic V(LDL) and has become a target to increase HDL-C.[92]

In an early study, antisense oligonucleotides against CETP suppressed atherosclerosis with a reduction in LDL-C and a small increase in HDL-C in JW rabbits.[36] Several studies have confirmed a reduction of lesion development after treatment with anti-CETP vaccines in NZW rabbits[37-40] and small molecule inhibitors, torcetrapib and anacetrapib in NZW rabbits[43] and APOE*3Leiden.CETP mice.[44, 45] In the rabbit study, aortic lesion area correlated with TC/HDL-C ratio and a trend toward an inverse correlation between HDL-C and lesion size was found. It should be noted, however, that torcetrapib produced a pro-inflammatory, unstable plaque phenotype possibly related to an increase in aldosterone levels in APOE*3Leiden.CETP mice,[44] the latter is in line with data from clinical studies.[23] Inconsistent data have been reported on the atheroprotective effects of dalcetrapib in JW rabbits[41, 42] where a reduction in atherosclerosis was only found when accompanied by a decrease in non-HDL-C levels, the latter of which is in contrast to clinical findings.[41] In the study by Huang *et al.*, despite no effect of treatment on lesion size, TC and non-HDL-C, but not HDL-C correlated with lesion area.[42] In APOE*3Leiden.CETP mice treated with anacetrapib, HDL-C inversely correlated

with lesion area, however, only non-HDL-C and not HDL-C independently determined lesion size.[45]

4.1.4 Scavenger receptor B-I (SR-BI) inhibitor and ATP-binding cassette A1 (ABCA1) degradation inhibitors

SR-BI mediates selective uptake of HDL-cholesterol esters by the liver and cholesterol efflux from other tissues. The SR-BI inhibitor, ITX5061 increased HDL-C without increasing (V) LDL-C and reduced atherosclerotic lesion area in *ldlr*$^{+/-}$ mice expressing CETP.[46] ATP-binding cassette (ABC) transporter, ABCA1 plays an important role in cholesterol efflux by mediating cholesterol transport to lipid-poor apolipoprotein A-I.[10] In rabbits, pharmacological inhibition of ABCA1 degradation increased HDL-C and reduced atherosclerotic lesion area.[47]

4.2 Treatment strategies aimed at raising HDL-C and cardiovascular disease: randomized controlled clinical trials

The efficacy and cost-effectiveness of statins have assured its partaking in randomized controlled trials involving high risk patients. This has led to a noticeable decrease in baseline LDL-C when compared to previous trials. It is not surprising that in relevant clinical trials involving patients with a baseline LDL-C of < 100 mg/dL, on average 82% of the patients were on prior statin treatment. In a meta-analysis involving 8 statin trials and 38 153 participants, HDL-C and apolipoprotein A-I levels, as well as the increase in apolipoprotein A-I were associated with reduced cardiovascular risk, however no association was found for the increase in HDL-C.[22] This is in line with results from the current meta-analysis with other lipid-modulating therapies where we observed no association between between-group differences in absolute or percentage change from baseline in HDL-C and non-fatal myocardial infarction, whereas a trend toward an association between absolute change from baseline in LDL-C levels and non-fatal myocardial infarction was found.

4.2.1 The effect of treatment on clinical outcomes in patient populations with high baseline LDL-C

Niacin reduced non-fatal myocardial infarction, whereas clofibrate/clofibric acid did not protect against cardiovascular disease in coronary heart disease patients in the CDP trial [72] and in newly diagnosed diabetic patients in the DIS trial.[70] In the WHO trial, clofibrate decreased non-fatal myocardial infarction in healthy subjects.[71] LDL-C and HDL-C levels were not measured in these earlier trials. Gemfibrozil, a fibrate that decreases LDL-C and increases HDL-C, reduced non-fatal myocardial infarction in dyslipidemic patients in the HHS trial[66] and in coronary heart disease patients in the VA-HIT trial,[69] but failed to affect clinical outcomes in patients with suspected heart disease that were excluded from the original HHS trial.[67] The reason for failure in the latter trial was ascribed to lack of power and

heterogeneity. In the VA-HIT trial, baseline LDL-C levels were much lower as compared to other trials without statins whereby no decrease in LDL-C was reported during the trial. In the BIP trial, bezafibrate had favorable effects on both LDL-C and HDL-C, but the reduction in cardiovascular events did not reach significance in patients with coronary artery disease,[62] whereas bezafibrate significantly reduced non-fatal myocardial infarction in patients with lower extremity arterial disease in the LEADER trial.[68] The angiographic DAIS trial that investigated the effects of fenofibrate in diabetic patients was not designed to detect differences in events,[63] but showed comparable reductions in clinical endpoints to that of post-hoc analyses in subgroups with diabetes.[66, 69] In the FIELD trial, fenofibrate reduced non-fatal myocardial infarction in diabetic patients.[65] Fenofibrate decreased LDL-C in both trials, but the increase in HDL-C was not apparent at study closure in the FIELD trial. It should be noted that during the BIP, LEADER and FIELD trials, significantly more patients in the placebo group received lipid-modifying drugs, mostly statins. This could have contributed to the unexpected reduction in the cumulative probability of the primary endpoint after placebo treatment in the BIP trial, especially given the decline in LDL-C towards the end of the trial.

4.2.2 The effect of treatment on clinical outcomes in patient populations with low baseline LDL-C

The ACCORD and the FIRST trials were the only 2 trials that investigated the effects of a fibrate in combination with a statin in patients with diabetes[29] and dyslipidemia.[64] At the end of these trials, fenofibrate/fenofibric acid did not significantly affect LDL-C, had a small effect on HDL-C and failed to reduce cardiovascular outcomes. However, fenofibrate treatment in patient with high baseline TG and low baseline HDL-C appeared to be beneficial in post-hoc analysis.[29]

Despite a decrease in LDL-C and an increase in HDL-C, the lack of efficacy of niacin in cardiovascular disease patients ascribed to insufficient power led to the premature termination of the AIM-HIGH trial.[15] However, in the much larger HPS2-THRIVE study, niacin-laropiprant also failed to reduce the risk of cardiovascular events in high risk patients.[16] A potential adverse effect of laropiprant on the clinical outcome cannot not be fully excluded.

In the dal-OUTCOMES trial, dalcetrapib, a CETP inhibitor which only raises HDL-C without affecting LDL-C had no effect on cardiovascular events in patients with recent acute coronary syndrome and although not significant, the 0.6 mmHg rise in systolic blood pressure and 18% increase in C-reactive protein certainly warrants attention, specifically with regards to other CETP inhibitors currently in clinical development.[17] In the DEFINE trial, treatment with anacetrapib showed a non-significant 18% increase in C-reactive protein levels without affecting blood pressure and within the power limits of this trial, anacetrapib did not reveal similar adverse cardiovascular effects as torcetrapib.[73]

In aggregate, none of the studies with (reasonably) well-treated patients using statins showed additional beneficial effects on top of statin treatment with different classes of intervention (fenofibrate/fenofibric acid, niacin and dalcetrapib), perhaps with the exception of patients with high TG and low HDL levels at baseline. This indicates that using the current therapeutic options patients are treated well and that more powerful treatment modalities are needed to lower the residual cardiovascular risk.

4.2.3 The effect of treatment on clinical outcomes in trials excluded from the meta-analyses due to serious off-target cardiovascular adverse events

In the ILLUMINATE trial, torcetrapib favorably affected both LDL-C and HDL-C, but increased mortality most likely due to an off-target increase in aldosterone and blood pressure.[23] Interestingly, post-hoc analysis revealed lower risk of cardiovascular events in patients with a higher increase in HDL/apolipoprotein A-I from baseline to 1-3 month of treatment. Three imaging studies, the ILLUSTRATE, RADIANCE 1 and RADIANCE 2 trials also reported more serious clinical adverse events (cardiovascular and blood pressure-related events) after torcetrapib treatment in patients with coronary disease, familial hypercholesterolemia and dyslipidemia, although these trials were underpowered to detect differences in events.[24-26]

Pioglitazone and aleglitazar increased both LDL-C and HDL-C and non-significantly reduced nonfatal myocardial infarction in diabetic patients in the PROactive[27] and the AleCardio trials [28]. In both trials, however, more patients in the treatment group suffered from heart failure, indicating adverse off-target effects. Development of other PPAR-α/γ agonists, muraglitazar and tesaglitazar were also stopped due to adverse events.[93]

4.3 Novel treatment strategies specifically targeting HDL on atherosclerosis: preclinical studies and clinical trials

4.3.1 Purified, reconstituted and delipidated HDL

Badimon *et al*. demonstrated that administration of homologous HDL fraction not only inhibited aortic fatty streak formation, but also induced lesion regression in NZW rabbits [48]. Purified rabbit apolipoprotein A-I administration to NZW rabbits reduced aortic fatty streak progression without inducing regression.[50] In these studies, the lack of plasma lipid modification and the reduction in aortic lipid accumulation suggested a direct role of HDL and/or apolipoprotein A-I on the vessel wall, possibly via an increase in reverse cholesterol transport. However, injection of reconstituted HDL failed to protect against fatty streak development and did not reduce aortic cholesterol content in JW rabbits.[49] Results from a more recent study in NZW rabbits show that native or reconstituted HDL infusion reduced atherosclerotic lesion area and improved lesion stability to a similar extent as statins.[51]

In humans, infusions of reconstituted HDL, CSL-111 reduced atheroma volume from baseline, but not versus placebo in the ERASE trial,[94] whereas another small study revealed

a significant reduction in lipid content in the plaque after CSL-111 infusion versus placebo.[95] Both studies showed improved plaque characteristics. The reinfusion of delipidated plasma HDL, another potential approach to improve reverse cholesterol transport, non-significantly reduce atheroma volume in humans.[96]

4.3.2 Apolipoprotein A-I Milano

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,[97, 98] possibly due to accelerated binding and dissociation from lipids.[99] In NZW rabbits, short-term administration of apolipoprotein A-I Milano reduced atherosclerosis progression,[52, 53, 55] induced rapid regression and improved plaque stability.[54, 56, 57] Interestingly, apolipoprotein A-I Milano showed similar effects on aortic cholesterol content with a greater reduction in intimal macrophage content as compared to phospholipid carrier alone.[52] Other mechanisms besides reverse cholesterol transport were, therefore, suggested, including anti-oxidant, anti-inflammatory and vasodilatory effects. In another study, HDL Milano and HDL wild-type showed similar reductions in reverse cholesterol transport as evidenced by a reduction in aortic cholesterol content and up-regulation of ABCA1 and SRBI.[56]

In clinical trials, recombinant apolipoprotein A-I Milano, ETC-216 demonstrated rapid regression of atherosclerosis as seen by a reduction in atheroma volume from baseline[100] that was characterized by rapid remodeling with consequently no effect on lumen volume.[101]

4.3.3 Apolipoprotein mimetic peptides

In NZW rabbits, the apolipoprotein AI mimetic peptides, D-4F and L-4F decreased atherosclerotic lesion area and reported a greater predictive value of inflammation markers as opposed to HDL-C levels.[59] In WHHL rabbits, infusion of apolipoprotein A-I mimetic peptide/phospholipid complexes inhibited the progression of atherosclerosis mainly due to changes in LDL charge and by converting small, dense LDL into large, buoyant LDL.[58] According to our knowledge, the effects of other apolipoprotein A-I mimetic peptides, 6F and 5A, as well as an apolipoprotein E-derived HDL mimetic peptide, ATI-5261 were only investigated in animals lacking CETP.[102, 103]

In the CHI-SQUARE clinical trial, the HDL mimetic, CER-001 failed to reduce atheroma volume when compared with placebo and although not powered for clinical outcomes, revealed no differences in endpoints between groups.[104]

4.3.4 Apolipoprotein A-I inducer

In preclinical development, the effects of the apolipoprotein A-I inducer, RVX-208 on atherosclerosis were investigated in an animal model lacking CETP.[105]

In the ASSURE trial, RVX-208 failed to reduce atheroma volume in statin-treated patients.[103]

Based on the present data, reconstituted HDL and apolipoprotein A-I-based treatment strategies seem promising in the protection against atherosclerosis development and cardiovascular disease.

4.4 Limitations

To specifically investigate the role of non-HDL-C/LDL-C versus HDL-C, we pooled the effects of different compounds with different mechanisms of action. It is possible that these compounds also have different anti-atherosclerotic properties that are independent of their lipid-modifying effects. In addition, the studies that did not report lipid levels necessary to perform the analyses were excluded. Most compounds affected both LDL-C and HDL-C and it is, therefore, difficult to truly determine the contribution of each separate lipid fraction. In our preclinical studies with niacin[32] and anacetrapib,[45] we have tried to address this issue by performing statistical analyses (analysis of covariance) which suggested that anacetrapib mainly decreased atherosclerotic lesion development via a reduction in non-HDL-C, whereas the increase in HDL-C with niacin contributed to some extent to the reduction of atherosclerosis progression.

4.5 Current status and future perspectives

Niacin and fibrates have been clinically available for many years. If indeed the baseline LDL-C levels in recent clinical trials were too low to detect reductions in clinical outcomes and after careful consideration of the reported adverse events in these trials, niacin and fibrates may still be feasible treatment options for certain patient populations, such as statin-intolerant patients, patients with familial hypercholesterolemia and dysbetalipoproteinemia, and patients with different forms of hypertriglyceridemia. In fact, a more potent PPAR-α agonist, K-877 is currently being investigated in phase II/III clinical trials.[106] Despite failure of torcetrapib and dalcetrapib, the CETP inhibitors, anacetrapib and evacetrapib are currently being investigated in phase III clinical trials (the REVEAL and ACCELERATE trials) and TA-8995 (DEZ-001) is in phase II clinical development (clinicaltrials.gov).

Apolipoprotein A-I Milano (ETC-216, now MDCO-216) had manufacturing problems which limited its development.[107] However, the compound is still in development. Phase II trials investigating the effects of reconstituted HDL and apolipoprotein A-I mimetic peptide infusions (CSL-112, CER-001, APL-180 (L-4F)) are ongoing (clinicaltrials.gov). Other apolipoprotein mimetic peptides, 5A, 6F and ATI-5261 are currently in preclinical development.[102, 103] Another infusion therapy with recombinant human lecithin cholesterol acyltransferase (LCAT), ACP-501 recently passed a phase I trial (clinical trials.gov) and

was shown to increase HDL-C in patients with coronary artery disease.[103] LCAT esterifies cholesterol, thereby converting small lipid-poor nascent HDL into larger spherical HDL and may play a role in reverse cholesterol transport [102]. A small molecule activator of LCAT in hamsters increased HDL-C, HDL particle size, plasma apolipoprotein A-I level and plasma cholesteryl ester (CE) to free cholesterol ratio and significantly reduced VLDL-C.[108] In addition, phase II trials investigating the effects of an apolipoprotein A-I inducer (RVX208/ RVX000222) were recently completed (clinicaltrials.gov).

Other compounds in clinical development not yet discussed in this review due to lack of studies in CETP-expression animals or lack of efficacy (no increase in HDL-C), include PPAR-δ agonists, LXR agonists and miR-33 antagonism. Data from early phase II trials suggest that treatment with PPAR-δ agonists, GW501516 and MBX-8025 may be beneficial in patients with metabolic dysfunction.[60] The clinical development of agonists of the transcription factor LXR is hindered due to its undesired effects on de novo lipogenesis and induction of CETP expression. The LXR agonist, LXR-623 also revealed central nervous system-related adverse events in phase I.[102] Inhibition of miR33 improved ABCA1 expression and increased plasma HDL levels in preclinical studies and clinical trials should follow soon.[103] Additional HDL-targeting compounds in preclinical development include endothelial lipase inhibitors and antisense oligonucleotides targeting CETP.[10]

5. Conclusion

According to results from the current systematic review and meta-analysis, as well as supporting evidence obtained from the literature, we conclude that the protective role of lowering LDL-C and non-HDL-C is well-established, although occasionally LDL-C lowering compounds have failed due to (off-target) side effects. The contribution of raising HDL-C on inhibition of atherosclerosis and the prevention of cardiovascular disease remains undefined and may be dependent on the mode of action of HDL-C-modification. Nonetheless, treatment strategies aimed at improving HDL function and raising apolipoprotein A-I may be worth exploring.

Disclosures

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