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Effect of evolocumab on fasting and post fat load lipids and lipoproteins in familial dysbetalipoproteinemia

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KEYWORDS
Familial dysbetalipoproteinemia;
Proprotein convertase subtilin kexin 9;
Evolocumab;
Clinical trial;
Non-HDL-cholesterol

BACKGROUND: Familial dysbetalipoproteinemia (FD) is the second most common monogenic lipid disorder (prevalence 1 in 850-3500), characterized by postprandial remnant accumulation and associated with increased cardiovascular disease (CVD) risk. Many FD patients do not achieve non-HDL-C treatment goals, indicating the need for additional lipid-lowering treatment options.

OBJECTIVES: To evaluate the effect of the PCSK9 monoclonal antibody evolocumab added to standard lipid-lowering therapy on fasting and post fat load lipids and lipoproteins in patients with FD.

METHODS: A randomized placebo-controlled double-blind crossover trial comparing evolocumab (140 mg subcutaneous every 2 weeks) with placebo during two 12-week treatment periods. At the start and end of each treatment period patients received an oral fat load. The primary endpoint was the 8-hour post fat load non-HDL-C area under the curve (AUC). Secondary endpoints included fasting and post fat load lipids and lipoproteins.

RESULTS: In total, 28 patients completed the study. Mean age was 62±9 years and 93% had an Ɛ2Ɛ2 genotype. Evolocumab reduced the 8-hour post fat load non-HDL-C AUC with 49% (95%CI 42-55) and apolipoprotein B (apoB) AUC with 47% (95%CI 41-53). Other fasting and absolute post fat load lipids and lipoproteins including triglycerides and remnant-cholesterol were also significantly reduced by evolocumab. However, evolocumab did not have significant effects on the rise above fasting levels that occurred after consumption of the oral fat load.

CONCLUSIONS: Evolocumab added to standard lipid-lowering therapy significantly reduced fasting and absolute post fat load concentrations of non-HDL-C, apoB and other atherogenic lipids and lipopro-

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teins in FD patients. The clinically significant decrease in lipids and lipoproteins can be expected to translate into a reduction in CVD risk in these high-risk patients.

Introduction

Familial dysbetalipoproteinemia (FD), also known as 'remnant removal disease', is the second most common monogenic lipid disorder after heterozygous familial hypercholesterolemia (heFH), with an estimated prevalence of 1 in 850 to 1 in 3500 individuals.[1] In clinical practice, FD is often not recognized and therefore underdiagnosed. FD is characterized by the accumulation of cholesterol-enriched remnants of triglyceride-rich lipoproteins (TRLs). TRLs are atherogenic and causally related to cardiovascular disease (CVD). TRL accumulation in FD is particularly pronounced during the postprandial phase, which is associated with a very high risk of CVD,[2,3] therefore FD patients have a very high risk of premature CVD.[4-6] Accumulation of TRLs is reflected in increased non-high-density lipoprotein cholesterol (non-HDL-C) levels that consist of cholesterol in all atherogenic lipoproteins such as chylomicrons, very-low-density lipoproteins (VLDL), their remnants and low-density lipoprotein (LDL). In FD patients, LDL and LDL-cholesterol (LDL-C) levels are generally low or even absent,[7,8] and thus do not adequately reflect CVD risk. Also, LDL-C levels cannot be measured accurately and therefore should not be measured in FD. Therefore, treatment goals for patients with FD are based on non-HDL-C levels. Medical treatment with diet, statins, and optionally fibrates, are recommended to achieve non-HDL-C treatment goals. However, in clinical practice 60% of FD patients do not achieve non-HDL-C treatment goals, even with optimal therapy, indicating the need for more intensive lipid-lowering treatment.[4]

Proprotein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibodies (mAbs) neutralize circulating PCSK9 and thereby prevent degradation of the LDL-receptor (LDL-R). PCSK9 mAbs were shown to lower LDL-C by 50-60%[9,10] and reduce CVD risk with 20% in high-risk patients.[11,12] In patients with type 2 diabetes mellitus (T2DM) PCSK9 mAbs effectively lower postprandial TRLs by approximately 30-40%.[13-16] The present study was designed to investigate whether the effect of PCSK9 mAbs would be similar in FD patients, since they generally have low LDL-C levels and TRLs with dysfunctional apoE that does not bind to the LDL-R. The aim of the EVOLVE-FD (Effects of EVOLocumab VErsus placebo added to standard lipid-lowering therapy on fasting and post fat load lipids in patients with familial dysbetalipoproteinemia) study was to evaluate the effect of evolocumab 140 mg every 2 weeks added to standard lipid-lowering therapy on fasting and post fat load lipids and lipoproteins in patients with FD.

Methods

The EVOLVE-FD trial was an investigator-initiated study conducted at four University Medical Centers (University Medical Center Utrecht, Erasmus University Medical Center Rotterdam, Amsterdam University Medical Center, Radboud University Medical Center Nijmegen) in the Netherlands. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. The study was approved by the Medical Ethics Review Committee of the UMC Utrecht and by the competent authority of the Netherlands. All patients provided written informed consent before study procedures were initiated.

Patients

Patients diagnosed with FD between 18 and 80 years of age, were eligible for study participation. A FD genotype (an ε2ε2 genotype or a pathogenic dominant *APOE* variant known to associate with a FD phenotype) confirmed by genotyping or isoelectric focusing was required. FD lipid phenotype was defined as either apoB/total cholesterol (TC) ratio <0.39 mg/dL [<0.15 mmol/g],[17] TC >193 mg/dL [>5 mmol/L] and triglycerides (TG) >266 mg/dL [>3 mmol/L],[18] or non-HDL-C/apoB >1.43 mg/dL [>3.69 mmol/g],[19] with or without lipid-lowering medication. If patients were using lipid-lowering medication the dose must have been stable for at least three months and non-HDL-C levels had to be >62 mg/dL [>1.6 mmol/L]. A complete list of in- and exclusion criteria is available in the Supplementary methods.

Study design and study drug

EVOLVE-FD was a multicenter, double-blind, placebo-controlled, crossover study (Fig. 1). Patients received subcutaneous auto-injectors of evolocumab 140 mg or auto-injectors with matching placebo every 2 weeks during 2 periods of 12 weeks in a random order (both provided by Amgen, Breda, the Netherlands). Between the 2 treatment periods the washout period of 8 weeks without study medication to prevent carryover effects. This duration was chosen because the estimated half-life of evolocumab is 11-17 days.[20] After the second 12-week treatment period there was a run-out period of 8 weeks to assess any potential adverse events. Randomization for treatment order was based on computer generated randomization with variable block size, stratified for participating center. Patients and staffs were blinded for treatment and outcome measures throughout the study.

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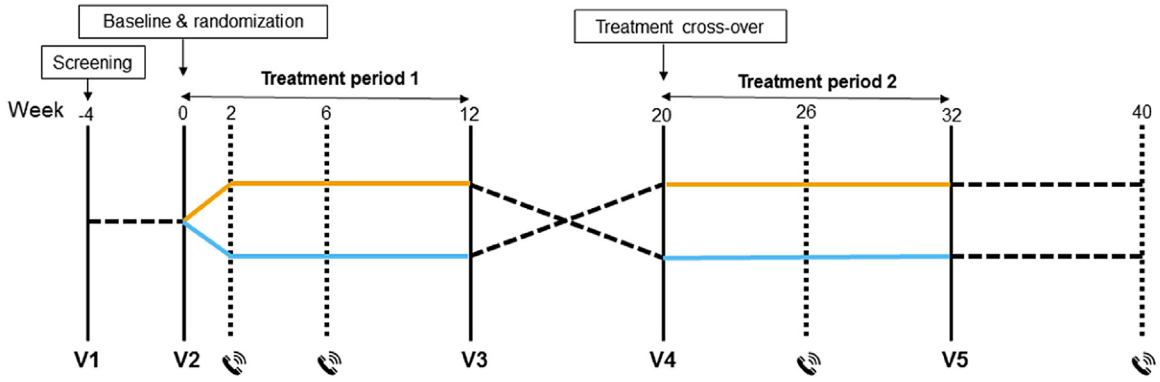

Figure 1 Cross-over study design.
At visit 2, 3, 4 and 5 an oral fat load was given and during 8 hours blood was drawn. Patients were randomized to treatment order, meaning that all patients used both evolocumab and placebo during the study. At week 2, 6, 26 and 40 there were phone calls to assess adherence to study medication, the injecting procedure and potential adverse events. Depending on randomization order, patients received evolocumab (orange) and placebo (blue) in the first or second treatment period.

Study procedures, oral fat load and data collection

At every visit, each patient underwent a standardized protocol including measurements of anthropometric characteristics, blood pressure and pulse. Use of medication, consumption of alcohol, smoking and physical activity were recorded. Potential adverse events were recorded and physical examination was performed. Patients were instructed not to change their diet, alcohol use, physical activity or dose and type of standard lipid-lowering medication during the study. At the start and at the end of both 12-week treatment periods, patients visited the hospital after a $\geq$12 hour overnight fast and received an oral fat load. The oral fat load consisted of unsweetened fresh cream (Albert Heijn, Zaandam, the Netherlands). Per 100 mL the fat load consisted of 1389 kJ (337 kcal), 35 gr fat (saturated 24 gr, unsaturated 11 gr), carbohydrates 3 gr, protein 2.5 gr), salt 0.1 gr). Cream was administered at a dose of 110 gr of fat per square meter of body surface area, with a maximum of 500 mL and ingested within a 10-minute time period. Cream was chosen to challenge the metabolic system with an extreme intake of fat and because it is a standardized product. Before and 1, 2, 4, 6 and 8 hours after the oral fat load venous blood samples were collected. During these 8 hours patients were not allowed to eat or to drink (except water). Further methods including definitions and (laboratory) measurements are provided in the Supplementary methods.

Primary and secondary study endpoints

The primary study endpoint was the difference in the 8-hour post fat load area under the curve (AUC) in non-HDL-C after treatment of 12 weeks evolocumab compared to placebo. We chose non-HDL-C since this is the treatment goal for FD patients and the best clinically available parameter and a reliable proxy for atherosclerosis risk in FD. The AUC was chosen to express the primary endpoint because it reflects the total exposure of atherogenic lipoproteins in FD after an oral fat load. Furthermore, the AUC predicts CVD events.[21] Secondary endpoints were fasting levels, post fat load exposure (8-hour post fat load AUC) and post fat load response (8-hour post fat load incremental AUC (iAUC)) of non-HDL-C, TC, TG, apoB, HDL-C, VLDL-C, remnant cholesterol (remnant-C), and fasting lipoprotein(a) (Lp(a)). The samples were analyzed in a central clinical laboratory practicing quality control for these analyses. The cholesterol content in the VLDL fraction (density range $0.94<d<1.006$ g/mL) and IDL fraction (density range $1.006<d<1.019$ g/mL) was measured with density gradient ultracentrifugation.[22] Remnant-C was defined as cholesterol in the IDL fraction. A detailed outline of all laboratory techniques is provided in the Supplementary methods.

The proportion of patients who achieved their non-HDL-C treatment goals was assessed. Non-HDL-C treatment goals in FD are defined as $<$131 mg/dL [$<$3.4 mmol/L] for FD patients without CVD and $<$100 mg/dL [$<$2.6 mmol/L] for FD patients with established CVD or T2DM, according to European guidelines for patients with increased TG.[23]

The safety of evolocumab was assessed through adverse event reporting and safety laboratory measurements. Adverse events for placebo and evolocumab were reported over a 20-week period, including the 12-week treatment period and the subsequent 8-week wash-out period.

Power calculation

The sample size was based on an expected reduction of 8-hour post fat load AUC non-HDL-C by evolocumab of 25% compared to placebo, which was based on a previous meta-analysis that showed a 56.1% reduction in fasting non-HDL-C by evolocumab.[24] Based on the working mechanism of evolocumab, this finding was expected to consist largely of LDL-C reduction. Patients with FD have no or little LDL-C[7,8] and therefore a conservative, but clinically relevant, 25% reduction in non-HDL-C was chosen. With a power of 85% and an alpha of 5%, 74 evaluable subjects were needed in a parallel study. For a crossover design this sample size could

be reduced by 65% due to within-person controls ((1-rho)/2, with rho 0.3).[25] Therefore the required sample size for the study was 74*0.35=26 subjects that completed the study.

Data analyses

The 8-hour post fat load lipids and lipoproteins exposure and 8-hour postprandial response were expressed as AUC and iAUC, respectively. AUC was calculated with the trapezoidal rule. The iAUC was calculated after adjustment for fasting lipid levels by subtracting eight (hours)*(value at time point 0) from the AUC (Supplementary Fig. 1). Absolute and percentage difference between two treatment arms for every patient were calculated and, to obtain robust confidence intervals (CIs) with corresponding p-values, CIs were computed by bootstrapping (1000 samples with replacement). Also, baseline lipid concentrations were taken into account. In these analyses the differences in change from baseline after treatment with evolocumab and placebo were compared. Carryover and period effects were assessed with an independent samples *t*-test. No carryover (p=0.65) or period effects (p=0.13) were observed. All clinical variables at baseline were complete, except for waist circumference (n=5). All lab variables were complete except for one apoB measurement at a single post fat load time point. Missing values were imputed with last observation carried forward. P <0.05 was considered statistically significant. All analyses were performed with RStudio, version 3.5.1.

Results

Patient disposition

Thirty-six patients were screened, and 31 patients were randomized. Reasons for screening failure were severe dyslipidemia requiring initiation of lipid-lowering treatment first and not having an *APOE* genotype that was associated with FD. During follow-up, there was 1 withdrawal of informed consent and 2 dropouts, because they did not complete all (post fat load) measurements to assess the primary endpoint, making 28 patients eligible for the analyses (Fig. 2). Details on reasons for screening failure, withdrawal and dropout are shown in Supplementary Table 1 and baseline characteristics of patients who withdrew consent or dropped out are shown in Supplementary Table 2. There were no clinically relevant differences at baseline between the patients in- and excluded in the efficacy analysis.

Baseline characteristics

The mean age of the 28 FD patients who completed the study was 62 ± 9 years and 43% were women (Table 1). The majority (93%) of the patients had an ε2ε2 genotype, two patients had a pathogenic dominant variant in their *APOE* gene known to be associated with FD (apoE3-Leiden and p.Arg180His and one patient had an ε2ε2 genotype in combination with a dominant variant in *APOE* (p.Gly171Asp). Twenty-five percent of patients had a history of CVD, 32% had T2DM and 75% fulfilled the criteria for metabolic syndrome using the NCEP ATP III criteria at baseline.[26] Almost all (93%) patients used lipid-lowering medication, mostly a combination of a statin and ezetimibe (29%) or a statin and a fibrate (29%). Two patients were not taking lipid-lowering medication, one had mild dyslipidemia and was not taking lipid-lowering medication yet, another patient preferred to use red yeast rice only. This patient stopped using red yeast rice prior and during the study. High-intensity statins were used by 25% of the study population. Mean baseline non-HDL-C was 139 ± 66 mg/dL [3.6 ± 1.7 mmol/L] and mean baseline TG were 275 ± 168 [3.1 ± 1.9 mmol/L]. The baseline table stratified for treatment group is provided in Supplementary Table 3.

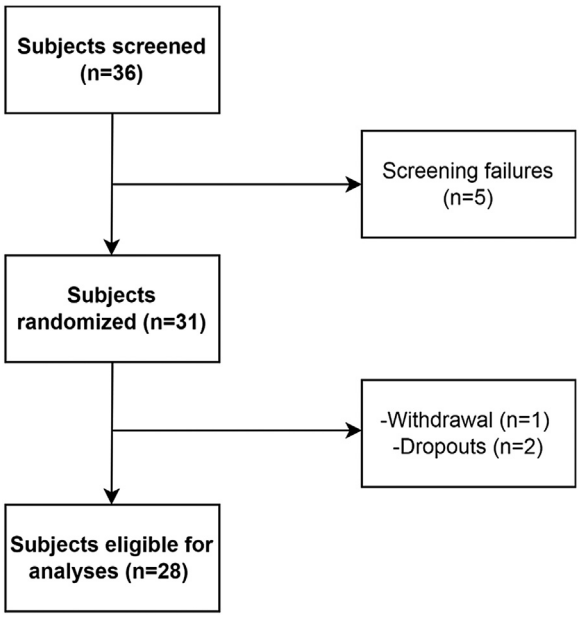

Figure 2 Patient disposition.
In total, 31 patients were randomized. There was 1 withdrawal and there were 2 dropouts, resulting in 28 patients eligible for analyses. Detailed information on reasons for screening failure, withdrawal and dropout, as well as baseline information for the 3 randomized patients who did not finish the study are provided in the Supplementary Table 1 and 2.

Fasting lipids and lipoproteins

Compared with placebo the mean absolute reduction in fasting non-HDL-C levels after 12 weeks evolocumab was 75 ± 44 mg/dL [1.9 ± 1.1 mmol/L], corresponding to a 51% (95%CI 43 – 57) relative reduction. With the exception of HDL-C, compared with placebo all fasting lipids and lipoproteins were significantly reduced after 12 weeks treatment with evolocumab. Compared with placebo the absolute reduction in fasting apoB levels after 12 weeks evolocumab was 96 ± 140 mg/dL [1.1 ± 1.6 mmol/L], corresponding to

Table 1 Baseline characteristics.

	Patients (n=28)
Age (years)	62 ± 9
Female sex	12 (43)
APOE genotype	
- ε2ε2	26 (93)
- Dominant *APOE* variant	3 (11)
Cardiovascular disease	7 (25)
- Coronary heart disease	2 (7)
- Peripheral vascular disease	1 (4)
- Cerebrovascular disease	3 (11)
- Abdominal aortic aneurysm	1 (4)
Diabetes mellitus type 2	9 (32)
Hypertension	22 (79)
Metabolic Syndrome	21 (75)
Family history of premature CVD	7 (25)
Lipid-lowering treatment	26 (93)
- Statin only	6 (21)
- Ezetimibe only	2 (7)
- Fibrate only	1 (4)
- Statin + ezetimibe	8 (29)
- Statin + fibrate	8 (29)
- Statin + ezetimibe + fibrate	1 (4)
High intensity statin	7 (25)
Current smoking	1 (4)
Body mass index (kg/m^2)	29.5 ± 3.6
Waist circumference (cm)	107 ± 11
Systolic blood pressure (mmHg)	141 ± 15
Diastolic blood pressure (mmHg)	85 ± 8
Laboratory measurements	
- Total cholesterol (mg/dL [mmol/L])	189 ± 73 [4.9 ± 1.9]
- Triglycerides (mg/dL [mmol/L])	275 ± 168 [3.1 ± 1.9]
- Non-HDL-cholesterol (mg/dL [mmol/L])	139 ± 66 [3.6 ± 1.7]
- HDL-cholesterol (mg/dL [mmol/L])	50 ± 15 [1.3 ± 0.4]
- Apolipoprotein B (mg/dL [g/L])	80 ± 20 [0.8 ± 0.2]
- Lipoprotein (a)* (mg/dL [mg/L])	8.2 (3.3 – 31.2) [82 (33 - 312)]
- Glucose (mg/dL [mmol/L])	110 ± 27 [6.1 ± 1.5]

Data are shown as n (%), mean ± standard deviation, or when not-normally distributed as median (interquartile range), indicated by *
Abbreviations: CVD = cardiovascular disease.

a 27% (95%CI 17 – 36) relative reduction. The mean relative reduction in fasting apoB was 48% (95%CI 42 – 53), in fasting VLDL-C 42% (95%CI 29 – 53) and in fasting remnant-C 44% (95%CI 30 – 55). Also, compared to placebo the median absolute reduction in fasting Lp(a) levels after treatment with evolocumab was 3.4 (IQR 0.1 – 13) mg/dL, corresponding to a 35% (95%CI 16 – 42) relative reduction (Table 2 and Fig. 3). The results were similar when taking the baseline measurements into account by comparing the difference in *change* from baseline in fasting lipids and lipoproteins (Supplementary Table 4 and Supplementary Fig. 3).

Post fat load lipids and lipoproteins

Compared with placebo the mean absolute reduction in 8-hour post fat load non-HDL-C exposure after 12 weeks evolocumab was 590 ± 352 mg/dL.8h [15.3 ± 9.1 mmol/L.8h], corresponding to a 49% (95%CI 42 – 55) relative reduction (Fig. 3 and Supplementary Fig. 5). Compared to placebo the mean percentage reduction in 8-hour post fat load TG after evolocumab was 20% (95%CI 10 – 29). Also, the mean reduction in 8-hour post fat load apoB exposure was 47% (95%CI 41 – 53). Eight hour post fat load levels of the other lipids and lipoproteins, including VLDL-C (45% (95%CI 32 – 55) and remnant-C (49% (95%CI 38 – 59), were significantly reduced, except for HDL-C (3.4% (95%CI -8.5 – 2.1)) (Fig. 3 and Table 3).

There were no differences between evolocumab and placebo in the iAUC (postprandial response) during the 8 hours after the oral fat load for any of the lipids and lipoproteins (Supplementary Table 5). For all atherogenic lipoproteins, the reduction in AUC was primarily based on a reduction in fasting concentrations, rather than a change in iAUC (Supplementary Fig. 2). The 8-hour post fat load results were similar when taking baseline measurements into account by comparing the difference in *change* from baseline (Supple-

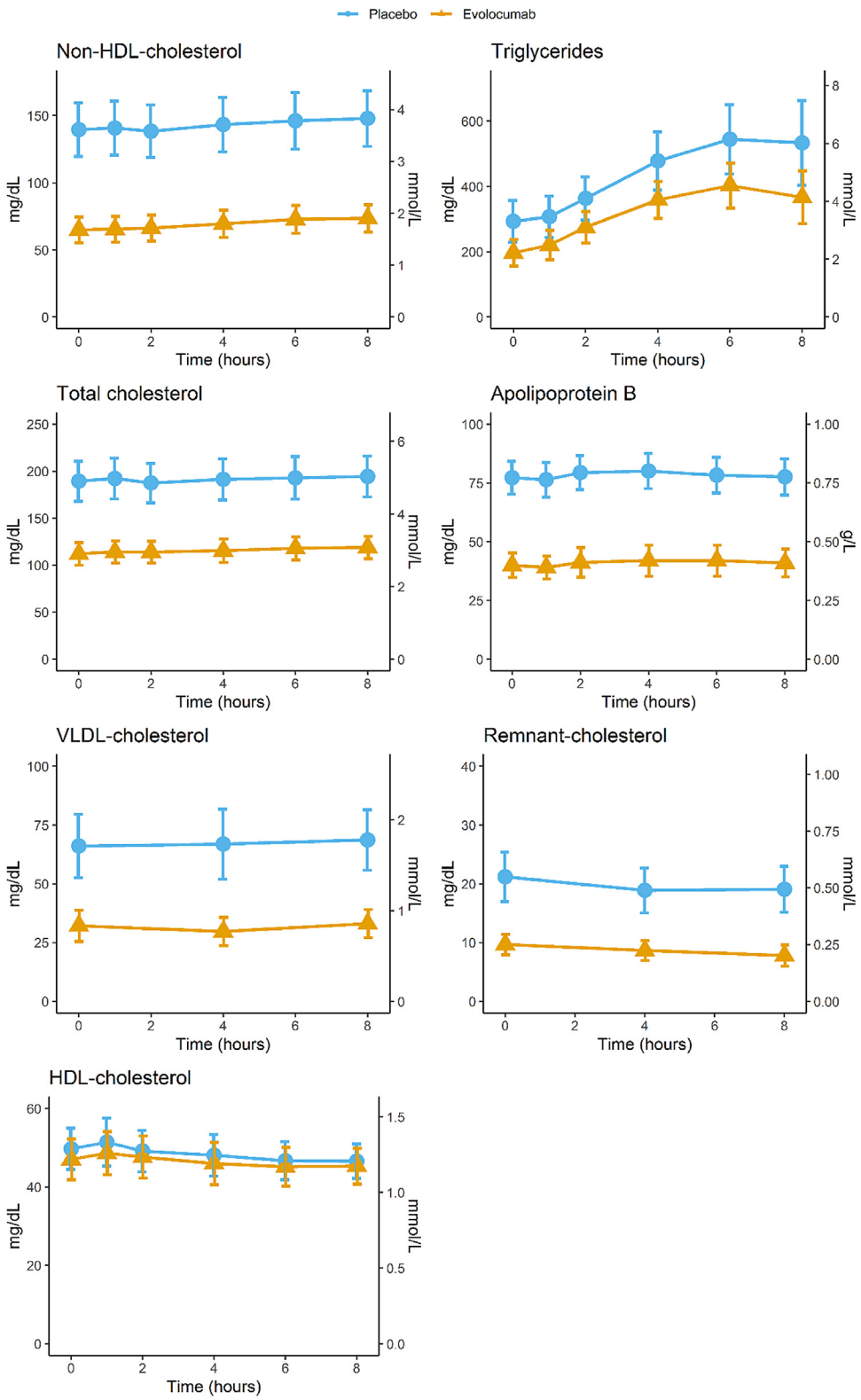

Figure 3 Effect of evolocumab and placebo on fasting and post fat load lipids and lipoproteins.
Fasting and 8-hour post fat load lipid and lipoprotein levels after an oral fat load, after treatment with evolocumab (orange) or placebo (blue).

Table 2 Effect of 12 weeks evolocumab compared to placebo on fasting lipids.

		After placebo	After evolocumab	Difference	% Difference (95%CI)	*P*-value
Non-HDL-C	mg/dL	140 ± 54	65 ± 26	-75 ± 44	-51 (-57 – -43)	<0.001
	mmol/L	3.6 ± 1.4	1.7 ± 0.7	-1.9 ± 1.1		
Triglycerides	mg/dL	293 ± 173	197 ± 110	-96 ± 140	-27 (-36 – -17)	<0.001
	mmol/L	3.3 ± 2.0	2.2 ± 1.2	-1.1 ± 1.6		
Total cholesterol	mg/dL	189 ± 57	112 ± 32	-77 ± 46	-39 (-45 – -32)	<0.001
	mmol/L	4.9 ± 1.5	2.9 ± 0.8	-2.0 ± 1.2		
apoB	mg/dL	77 ± 19	40 ± 14	-37 ± 17	-48 (-53 – -42)	<0.001
	g/L	0.8 ± 0.2	0.4 ± 0.1	-0.4 ± 0.2		
VLDL-C	mg/dL	66 ± 36	32 ± 18	-34 ± 34	-42 (-53 – -29)	<0.001
	mmol/L	1.7 ± 0.9	0.8 ± 0.5	-0.9 ± 0.9		
Remnant-C	mg/dL	21 ± 11	10 ± 5	-12 ± 10	-44 (-55 – -30)	<0.001
	mmol/L	0.5 ± 0.3	0.3 ± 0.1	-0.3 ± 0.3		
HDL-C	mg/dL	50 ± 14	47 ± 14	-2.7 ± 7.4	-4.3 (-10 – 3.0)	0.20
	mmol/L	1.3 ± 0.4	1.2 ± 0.4	-0.1 ± 0.2		
Lp(a)*	mg/dL	7.2 (3.1 – 35)	3.7 (3.0 – 22)	-3.4 (-13 – -0.1)	-35 (-42 – -16)	<0.001
	mg/L	72 (31 – 353)	37 (30 – 216)	-34 (-132 – -1)		

Values are mean ± standard deviation.

** Shown as medians (interquartile range) and percentage difference shown as median (95% confidence interval).Abbreviations: ApoB = apolipoprotein B, AUC = area under the curve, HDL-C = high-density lipoprotein-cholesterol, Lp(a) = Lipoprotein, Non-HDL-C = high-density lipoprotein-cholesterol, remnant-C = remnant-cholesterol, VLDL-C = very-low density lipoprotein-cholesterol.

Table 3 Effect of 12 weeks evolocumab compared to placebo on 8-hour post fat load lipids.

		AUC after placebo	AUC after evolocumab	Difference	% Difference (95%CI)	*P*-value
Non-HDL-C	mg/dL.8h	1145 ± 438	555 ± 215	-590 ± 352	-49 (-55 – -42)	<0.001
	mmol/L.8h	29.7 ± 11.3	14.4 ± 5.6	-15.3 ± 9.1		
Triglycerides	mg/dL.8h	3579 ± 1878	2623 ± 1209	-956 ± 1428	-20 (-29 – -10)	<0.001
	mmol/L.8h	40.4 ± 21.2	29.6 ± 13.6	-10.8 ± 16.1		
Total cholesterol	mg/dL.8h	1531 ± 470	926 ± 260	-605 ± 362	-38 (-43 – -31)	<0.001
	mmol/L.8h	39.7 ± 12.2	24.0 ± 6.7	-15.7 ± 9.4		
apoB	mg/dL.8h	629 ± 157	329 ± 130	-296 ± 133	-47 (-53 – -41)	<0.001
	g/L.8h	6.3 ± 1.6	3.3 ± 1.3	-3.0 ± 1.3		
VLDL-C	mg/dL.8h	537 ± 296	249 ± 129	-288 ± 273	-45 (-55 – -32)	<0.001
	mmol/L.8h	13.9 ± 7.7	6.5 ± 3.3	-7.5 ± 7.1		
Remnant-C	mg/dL.8h	156 ± 83	70 ± 36	-87 ± 69	-49 (-59 – -38)	<0.001
	mmol/L.8h	4.0 ± 2.1	1.8 ± 0.9	-2.2 ± 1.8		
HDL-C	mg/dL.8h	386 ± 111	371 ± 111	-15 ± 48	-3 (-9 – 2)	0.21
	mmol/L.8h	10.0 ± 2.9	9.6 ± 2.9	-0.4 ± 1.3		

Values are mean ± standard deviation.

Abbreviations: ApoB = apolipoprotein B, AUC = area under the curve, HDL-C = high-density lipoprotein-cholesterol, Non-HDL-C = high-density lipoprotein-cholesterol, remnant-C = remnant-cholesterol, VLDL-C = very-low density lipoprotein-cholesterol.

mentary Tables 6 and 7 and Supplementary Figs. 3 and 4). The individual responses for non-HDL-C and triglycerides after evolocumab and placebo are provided in Supplementary Fig. 6.

Non-HDL-C treatment goals

After 12 weeks treatment with evolocumab added to regular lipid-lowering treatment 89% of patients achieved their non-HDL-C treatme nt goal (<131 mg/dL [<3.4 mmol/L] or <100 mg/dL [<2.6 mmol/L]) compared with 36% after placebo. Moreover, 54% of the patients achieved a >50% reduction in non-HDL-C after 12 weeks treatment with evolocumab compared to none after placebo (Fig. 4).

Adverse events

In total, 75 adverse events occurred in 20 of the 31 patients that were randomized and received ≥1 dose of the study drug

Table 4 Overview of adverse events.

	During treatment with placebo Number of subjects (total AEs)	During treatment with evolocumab Number of subjects (total AEs)
Adverse events		
- Any	17 (30)	13 (45)
Most common adverse events		
- Nausea	3 (5)	4 (5)
- Myalgia	3 (4)	3 (4)
- Diarrhea	2 (3)	4 (4)
- COVID-19	1 (1)	4 (4)
- Arthralgia	1 (1)	2 (3)
Related to study drug		
- Injection site reaction	0 (0)	1 (1)
Serious		
- Any	0 (0)	4 (7)*

Values are n.

*In total seven SAEs occurred in four patients. The first patient was admitted to the hospital due to complications after an elective colonoscopy (1). The second patient was admitted due to complications after an elective cholecystectomy (2). A few days later the patient was readmitted after a bile leak after the cholecystectomy (3). The third patient was admitted to the hospital due to complication of a COVID-19 infection (4). The fourth patient was hospitalized for a coronary artery bypass graft surgery and aorta valve replacement (5). A few days later this patient was readmitted because of intermittent atrial fibrillation (6) and heart failure (7).

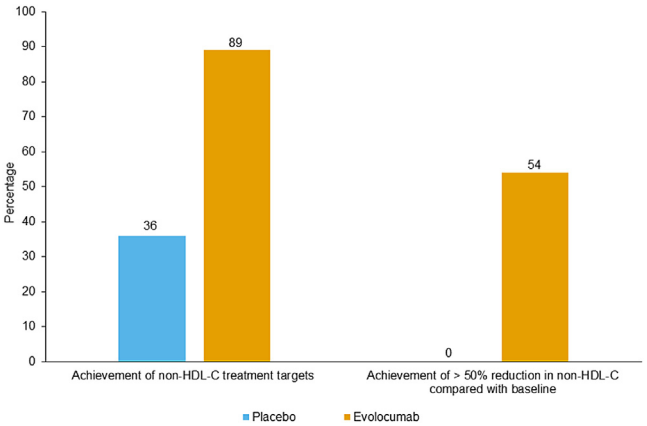

Figure 4 Achievement of treatment goals after evolocumab compared with placebo.

Achievement of treatment goals (non-HDL-C levels of <131 mg/dL [<3.4 mmol/L] or <100 mg/dL [<2.6 mmol/L] when CVD or T2DM at baseline) after treatment with evolocumab, compared with placebo.

Abbreviations: Non-HDL-C = non-high-density lipoprotein cholesterol.

(evolocumab or placebo). In total 30 adverse events (in 13 patients) during treatment with placebo and 45 (in 17 patients) during treatment with evolocumab were reported. Most adverse events were mild and temporary. One adverse event classified as definitely related to study drug and concerned a reaction at the injection site. In general, most common adverse events were gastro-intestinal complaints, muscle complaints and COVID-19 infection. An overview of adverse and serious adverse events is provided in Table 4 and Supplementary Table 8.

Discussion

In this randomized clinical trial in patients with genetically established FD, 12 weeks treatment with evolocumab compared to placebo, added to regular care, resulted in significant and clinically relevant reductions in fasting and absolute post fat load lipid and lipoprotein levels following an oral fat load. However, the postprandial rise 8 hours after an oral fat load remained unaffected by evolocumab. Almost all (89%) patients achieved their non-HDL-C treatment goals after treatment with evolocumab.

The main working mechanism of PCSK9 mAbs is increasing the number of LDL-R on the hepatocyte surface through inhibition of PCSK9. In FD the affinity of TRLs for the LDL-R is severely decreased (<2%) and FD patients usually have no or little LDL due to impaired lipolysis of VLDL remnants, which is thought to require functional apoE.[7,8] Furthermore, in patients with homozygous FH (hoFH) without residual LDL-R activity (defined as <2% residual activity for the LDL-R), PCSK9 mAbs have not been observed to have an effect on lipid levels.[27,28] Therefore, it was unknown whether PCSK9 mAbs could play a role in reducing atherogenic remnant lipoproteins in FD patients. A small observational non-randomized and unblinded study showed a reduction of 42% in fasting non-HDL-C and 44% in VLDL-C after PCSK9 mAbs for 12 weeks in 3 patients with FD.[29] This is line with our findings showing that PCSK9 mAbs are able to lower atherogenic lipids and lipoproteins in FD.

Several other studies investigated the effects of PCSK9 mAbs in populations with mixed dyslipidemias from other causes, such as T2DM.[13-16] Although patients with T2DM have an intact binding of apoE to the LDL-R, their lipoprotein phenotypes are somewhat similar to FD. An exploratory

analysis in 57 patients with T2DM showed that 3-hour post fat load non-HDL-C AUC after a mixed-meal were reduced with 43% compared to baseline after treatment with PCSK9 mAbs.[13] Fasting non-HDL-C was also significantly reduced with 46-56% in that study. In addition, that and other studies in patients with T2DM,[13-16] showed strikingly identical reductions in other lipid and lipoprotein fractions after treatment with PCSK9 mAbs (including TC, TG, apoB and VLDL-C) as seen in the present study in FD patients. This observation supports the idea that the effects of PCSK9 mAbs on TRLs may be, at least partly, independent of the binding of apoE to the LDL-R.[16]

Patients with FD have impaired clearance of TRLs, leading to increased and prolonged plasma concentrations of remnant lipoproteins in the postprandial phase.[3] In the present study, evolocumab reduced all atherogenic lipids and lipoproteins in the first 8 hours of the postprandial phase (AUC), that is attributable to the reduction in fasting levels. Therefore this study shows that, in FD, PCSK9 mAbs reduce fasting and absolute post fat load levels, but not the postprandial rise of TRLs. Postprandial lipemia is associated with a very high risk of CVD in non-FD patients and several prospective studies showed that elevated non-fasting TG plasma concentrations (as a marker for increased remnant-cholesterol) are associated with a 17-fold increase in the risk of myocardial infarction in women and 5-fold increase in men. Non-fasting TG concentrations are also associated with an increased risk for ischemic stroke and early death.[2,30] In FD, fasting TG values are higher than in the general population because of a delayed clearance of TRLs. This might explain why the difference in fasting and postprandial TRL levels was small in this study. Furthermore, in FD, CVD risk is probably more similarly reflected by both fasting and postprandial TRL levels. Therefore, the findings of this study can be expected to translate into a reduction in CVD risk.

How PCSK9 mAbs reduce lipid and lipoproteins in FD patients is not exactly known. Several hypotheses could be considered. First, a substantial increase in the number of LDL-Rs could have a lipid and lipoprotein lowering effect, even when TRLs have a severely reduced affinity for the LDL-R. In line with this, in our study there were two patients with a dominant variant in the *APOE* gene (with a higher affinity of apoE to the LDL-R receptor compared to the ε2ε2 genotype)[31] and both patients had an above average response to PCSK9 inhibition (Supplementary Fig. 4).

Second, PCSK9 mAbs might not only inhibit PCSK9, but also other members of the proprotein convertase (PC) family (such as furin, PCSK5, PCSK6 or PCSK7). These other PCs stimulate the cleavage of angiopoietin-like protein (ANGPTL) 3, 4 and/or 8,[32] thereby inhibiting the activity of lipoprotein lipase and endothelial lipase. Inhibition of these pathways by PCSK9 mAbs may lead to increased lipolysis and remodeling of TRLs, resulting in smaller remnants that are more rapidly and efficiently cleared by other hepatic clearance receptors. However, a major effect of PCSK9 mAbs on ANGPTL3/4 activity is unlikely in view of the absence of an LDL-C lowering effect in hoFH without residual LDL-R activity; a hallmark of ANGPTL3 inhibition.[27,28]

Third, TRLs are not exclusively cleared by the LDL-R. An important LDL-R independent system for clearance of TRLs is the LDL-R-related protein 1 (LRP1) the low-affinity, high-capacity heparin sulfate proteoglycan (HSPG) system.[33] There are several indications that PCSK9 mAbs upregulate receptors related to the LDL-R, including the LRP1, VLDL-R and apoE2 receptor. However, since the latter two receptors are not located in the liver it is thought that they play a limited role in the clearance of TRLs and are less sensitive to the effect of PCSK9 inhibition.[34] Although LDL-R-related protein 1 (LRP1) does play a significant role in TRL clearance, some studies in vitro and mice have been shown that this receptor is not degraded by PCSK9 overexpression,[35,36] but others do show an effect of PCSK9[37] or PCSK9 mAbs[38] on the LRP1. Furthermore, it is known that HSPGs help to present PCSK9 protein to the LDL-R,[39] but the effect of PCSK9 mAbs on the expression or function of HSPGs remains unknown. Stable isotope studies with labeled TRLs could provide further insight into the LDL-R independent mechanisms of action of PCSK9 mAbs.

In the present study 89% of patients attained their non-HDL-C treatment goals after treatment with evolocumab. After placebo this was 36%, which is in line with the 40% that was found in an observational cross-sectional study in 305 FD patients in the pre-PCSK9 era.[4]

In this study it was found that evolocumab had a good safety profile (Supplementary Table 9). FD patients often use a combination of lipid-lowering medication such as statins, fibrates and ezetimibe. In the present study the rate of adverse events by PCSK9 mAbs on top of these lipid-lowering medications was similar as reported in a meta-analysis assessing the safety of PCSK9 mAbs in patients with dyslipidemia or CVD.[40]

Strengths and limitations

Strengths of the study include the largest group of FD patients ever investigated in a randomized trial, the well characterized population, the crossover trial design, the fact that fasting and post fat load lipids and lipoproteins were studied in a single study and that evolocumab was studied on top of a background of treatment with different (combinations of) lipid-lowering medication.

A potential limitation of this study is the measurement of lipid levels up to 8 hours after the oral fat load. This might not be sufficient to cover the complete post fat load response in patients with FD. Measuring the response up to 24 hours after an oral fat load would have provided more information on the late phase of post fat load clearance. However, we decided that the additional patient burden of a 24 hour fast did not outweigh the extra information this measurement would provide. Although there is no universal method to define or measure remnant-cholesterol, in the present study we defined ultracentrifugally measured IDL-cholesterol as remnant-cholesterol, which is based on density

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and can therefore include small VLDL or large LDL as well. Second, only 29% of the patients were using a combination of a statin and a fibrate at baseline; while 82% used a statin (alone or in combination). The European Society of Cardiology (ESC) guidelines advise a statin or, if the lipoprotein phenotype is dominated by high TGs, a fibrate and state that often a combination of statin and fibrate may be needed.[23] Therefore, there is currently no 'optimal' treatment strategy against which the PCSK9 mAb treatment could have been compared. Furthermore, PCSK9 mAbs lower atherogenic lipid levels to the same extent with or without statins.[41] This is not known for fibrates, but fibrates have limited effects on non-HDL-C levels. Therefore, it is assumed that the relative results of this study can be generalized to all FD patients, regardless of their background lipid lowering medication. Third, although there was a very consistent relative reduction of lipids and lipoproteins in study participants, it cannot be ruled out that dietary changes or illnesses during the study have influenced lipids and lipoprotein levels as FD patients are very sensitive to any changes in diet or weight. Of the five patients that got COVID-19 during the study, three patients used evolocumab and no patients used placebo (two patients had COVID-19 during wash-out or follow-up). Although this might have led to an overestimation of the effect of evolocumab, a comparison of the results of these three patients showed slightly higher non-HDL-C plasma levels when compared with the other patients (Supplementary Table 10). Fourth, not all patients achieved their pre-randomization baseline lipid values prior to the start of the second treatment period when using PCSK9 in the first period (Supplementary Fig. 7). Although this could theoretically be due to lingering effects of PCSK9 mAbs administered in the first treatment period, there were no cross-over effects (p=0.65) and mean lipid values at start of the second treatment period were not lower in patients who first received PCSK9 mAb and then placebo (Supplementary Fig. 5).

To conclude, evolocumab added to standard lipid-lowering therapy significantly reduced fasting and absolute post fat load concentrations of non-HDL-C, apoB and other atherogenic lipids and lipoproteins in FD patients. The large decrease in lipids and lipoproteins can be expected to translate into a reduction in CVD risk in this high-risk patient population.

Contribution Statement

- Britt Heidemann: Writing – original draft, Formal analysis, Visualization, Project administration, Investigation.
- Charlotte Koopal: Writing - review & editing, Supervision, Methodology.
- Jeanine Roeters van Lennep: Writing - review & editing, Recruitment of participants.
- Erik Stroes: Writing - review & editing, Recruitment of participants, Investigation.
- Niels Riksen: Writing - review & editing, Recruitment of participants, Investigation.
- Monique Mulder: Writing - review & editing, Laboratory analyses, Investigation.
- Leonie van Vark – van der Zee: Writing - review & editing, Laboratory analyses, Investigation.
- Dee Blackhurst: Writing - review & editing, Laboratory analyses, Investigation.
- David Marais: Writing - review & editing, Supervision, Methodology, Laboratory analyses.
- Frank Visseren: Writing - review & editing, Supervision, Methodology, Recruitment of participants, Investigation, Funding acquisition.

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ADM declares no conflict of interest.
FV declares no conflict of interest.
All authors approved the final version of the manuscript.

CRediT authorship contribution statement

Britt E. Heidemann: Writing – original draft, Formal analysis, Visualization, Project administration, Investigation. **Charlotte Koopal:** Writing – review & editing, Supervision, Methodology.

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Supplementary materials

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References

1. Koopal C, Marais AD, Visseren FL. Familial dysbetalipoproteinemia: an underdiagnosed lipid disorder. *Curr Opin Endocrinol Diabetes Obes*. 2017;24(2):133–139.
2. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA*. 2007;298(3):299–308.
3. Weintraub MS, Eisenberg S, Breslow JL. Different patterns of postprandial lipoprotein metabolism in normal, type IIa, type III, and type IV hyperlipoproteinemic individuals. Effects of treatment with cholestyramine and gemfibrozil. *The Journal of clinical investigation*. 1987;79(4):1110–1119.
4. Koopal C, Retterstol K, Sjouke B, Hovingh GK, Ros E, de Graaf J, et al. Vascular risk factors, vascular disease, lipids and lipid targets in patients with familial dysbetalipoproteinemia: a European cross-sectional study. *Atherosclerosis*. 2015;240(1):90–97.
5. Hopkins PN, Wu LL, Hunt SC. Brinton EA. Plasma triglycerides and type III hyperlipidemia are independently associated with premature familial coronary artery disease. *Journal of the American College of Cardiology*. 2005;45(7):1003–1012.
6. Paquette M, Bernard S, Baass A. Dysbetalipoproteinemia is associated with increased risk of coronary and peripheral vascular disease. *The Journal of clinical endocrinology and metabolism*. 2022.
7. Blom DJ, Byrnes P, Jones S, Marais AD. Non-denaturing polyacrylamide gradient gel electrophoresis for the diagnosis of dysbetalipoproteinemia. *J Lipid Res*. 2003;44(1):212–217.
8. Mahley RW, Huang Y, Rall Jr SC. Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia). Questions, quandaries, and paradoxes. *J Lipid Res*. 1999;40(11):1933–1949.
9. Toth PP, Worthy G, Gandra SR, Sattar N, Bray S, Cheng LI, et al. Systematic Review and Network Meta-Analysis on the Efficacy of Evolocumab and Other Therapies for the Management of Lipid Levels in Hyperlipidemia. *J Am Heart Assoc*. 2017;6(10).
10. Karatasakis A, Danek BA, Karacsonyi J, Rangan BV, Roesle MK, Knickelbine T, et al. Effect of PCSK9 Inhibitors on Clinical Outcomes in Patients With Hypercholesterolemia: A Meta-Analysis of 35 Randomized Controlled Trials. *J Am Heart Assoc*. 2017;6(12).
11. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. *N Engl J Med*. 2017;376(18):1713–1722.
12. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. *N Engl J Med*. 2018;379(22):2097–2107.
13. Rosenson RS, Daviglus ML, Handelsman Y, Pozzilli P, Bays H, Monsalvo ML, et al. Efficacy and safety of evolocumab in individuals with type 2 diabetes mellitus: primary results of the randomised controlled BANTING study. *Diabetologia*. 2019;62(6):948–958.
14. Burggraaf B, Pouw NMC, Arroyo SF, van Vark-van der Zee LC, van de Geijn GM, Birnie E, et al. A placebo-controlled proof-of-concept study of alirocumab on postprandial lipids and vascular elasticity in insulin-treated patients with type 2 diabetes mellitus. *Diabetes Obes Metab*. 2020;22(5):807–816.
15. Taskinen MR, Björnson E, Andersson L, Kahri J, Porthan K, Matikainen N, et al. Impact of proprotein convertase subtilisin/kexin type 9 inhibition with evolocumab on the postprandial responses of triglyceride-rich lipoproteins in type II diabetic subjects. *J Clin Lipidol*. 2020;14(1):77–87.
16. Taskinen MR, Björnson E, Kahri J, Söderlund S, Matikainen N, Porthan K, et al. Effects of Evolocumab on the Postprandial Kinetics of Apo (Apolipoprotein) B100- and B48-Containing Lipoproteins in Subjects With Type 2 Diabetes. *Arteriosclerosis, thrombosis, and vascular biology*. 2021;41(2):962–975.
17. Blom DJ, O'Neill FH, Marais AD. Screening for dysbetalipoproteinemia by plasma cholesterol and apolipoprotein B concentrations. *Clinical chemistry*. 2005;51(5):904–907.
18. Blom DJ, Byrnes P, Jones S, Marais AD. Dysbetalipoproteinaemia–clinical and pathophysiological features. *S Afr Med J*. 2002;92(11):892–897.
19. Paquette M, Bernard S, Blank D, Paré G, Baass A. A simplified diagnosis algorithm for dysbetalipoproteinemia. *J Clin Lipidol*. 2020.
20. Kasichayanula S, Grover A, Emery MG, Gibbs MA, Somaratne R, Wasserman SM, et al. Clinical Pharmacokinetics and Pharmacodynamics of Evolocumab, a PCSK9 Inhibitor. *Clin Pharmacokinet*. 2018;57(7):769–779.
21. Patsch JR, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, et al. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler Thromb*. 1992;12(11):1336–1345.
22. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest*. 1955;34(9):1345–1353.
23. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *European heart journal*. 2020;41(1):111–188.
24. Zhang XL, Zhu QQ, Zhu L, Chen JZ, Chen QH, Li GN, et al. Safety and efficacy of anti-PCSK9 antibodies: a meta-analysis of 25 randomized, controlled trials. *BMC Med*. 2015;13:123.
25. Koopal C, Marais AD, Westerink J, van der Graaf Y, Visseren FLJ. Effect of adding bezafibrate to standard lipid-lowering therapy on post-fat load lipid levels in patients with familial dysbetalipoproteinemia. A randomized placebo-controlled crossover trial. *J Lipid Res*. 2017;58(11):2180–2187.
26. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Curr Opin Cardiol*. 2006;21(1):1–6.
27. Raal FJ, Honarpour N, Blom DJ, Hovingh GK, Xu F, Scott R, Wasserman SM, Stein EA. TESLA Investigators. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2015;385(9965):341–350. doi:10.1016/S0140-6736(14)61374-X.
28. Blom DJ, Harada-Shiba M, Rubba P, Gaudet D, Kastelein JJP, Charng M-J, Pordy R, Donahue S, Ali S, Dong Y, Khilla N, Banerjee B, Banerjee P, Baccara-Dinet M, Rosenson RS. Efficacy and Safety of Alirocumab in Adults With Homozygous Familial Hypercholesterolemia: The ODYSSEY HoFH Trial. *J Am Coll Cardiol*. 2020;76(2):131–142. doi:10.1016/j.jacc.2020.05.027.
29. Waldmann E, Wu L, Busygina K, Altenhofer J, Henze K, Folwaczny A, et al. Effect of PCSK9 inhibition with evolocumab on lipoprotein subfractions in familial dysbetalipoproteinemia (type III hyperlipidemia). *PloS one*. 2022;17(3):e0265838.
30. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *Jama*. 2008;300(18):2142–2152.

31. Koopal C, Marais AD, Westerink J, Visseren FL. Autosomal dominant familial dysbetalipoproteinemia: A pathophysiological framework and practical approach to diagnosis and therapy. *J Clin Lipidol*. 2017;11(1):12–23 e1.
32. Lei X, Shi F, Basu D, Huq A, Routhier S, Day R, et al. Proteolytic processing of angiopoietin-like protein 4 by proprotein convertases modulates its inhibitory effects on lipoprotein lipase activity. *J Biol Chem*. 2011;286(18):15747–15756.
33. Mahley RW, Ji Z-S. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *Journal of Lipid Research*. 1999;40(1):1–16.
34. Poirier S, Mayer G, Benjannet S, Bergeron E, Marcinkiewicz J, Nassoury N, et al. The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. *J Biol Chem*. 2008;283(4):2363–2372.
35. Park SW, Moon YA, Horton JD. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. *J Biol Chem*. 2004;279(48):50630–50638.
36. Benjannet S, Rhainds D, Essalmani R, Mayne J, Wickham L, Jin W, et al. NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. *J Biol Chem*. 2004;279(47):48865–48875.
37. Canuel M, Sun X, Asselin MC, Paramithiotis E, Prat A, Seidah NG. Proprotein convertase subtilisin/kexin type 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1). *PloS one*. 2013;8(5):e64145.
38. Paciullo F, Petito E, Falcinelli E, Gresele P, Momi S. Pleiotropic effects of PCSK9-inhibition on hemostasis: Anti-PCSK9 reduce FVIII levels by enhancing LRP1 expression. *Thromb Res*. 2022;213:170–172.
39. Gustafsen C, Olsen D, Vilstrup J, Lund S, Reinhardt A, Wellner N, et al. Heparan sulfate proteoglycans present PCSK9 to the LDL receptor. *Nat Commun*. 2017;8(1):503.
40. Guedeney P, Giustino G, Sorrentino S, Claessen BE, Camaj A, Kalkman DN, et al. Efficacy and safety of alirocumab and evolocumab: a systematic review and meta-analysis of randomized controlled trials. *European heart journal*. 2019.
41. Robinson JG, Nedergaard BS, Rogers WJ, Fialkow J, Neutel JM, Ramstad D, et al. Effect of evolocumab or ezetimibe added to moderate- or high-intensity statin therapy on LDL-C lowering in patients with hypercholesterolemia: the LAPLACE-2 randomized clinical trial. *Jama*. 2014;311(18):1870–1882.

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