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A placebo-controlled proof-of-concept study of alirocumab on postprandial lipids and vascular elasticity in insulin-treated patients with type 2 diabetes mellitus

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Abstract

Aim: Type 2 diabetes mellitus (T2DM) is associated with an increased risk of cardiovascular disease (CVD) linked to atherogenic dyslipidaemia and postprandial hyperlipidaemia. Alirocumab, a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor, improves CVD risk by reducing the concentration of low-density lipoprotein-cholesterol (LDL-C). However, effects of PCSK9 inhibitors on other aspects of diabetic dyslipidaemia, particularly in the postprandial situation, are less clear.

Material and Methods: Twelve male patients with T2DM on an intensive insulin regimen completed a 6-week randomized, double-blind, placebo-controlled, proof-of-concept study. Participants received three biweekly dosages of subcutaneous alirocumab (150 mg) or placebo. Before and after the intervention, fasting and postprandial triglyceride (TG) plasma levels, apolipoprotein (apo) B48, lipoprotein composition isolated by ultracentrifugation, vascular function and markers of inflammation were evaluated.

Results: Alirocumab treatment reduced fasting plasma TG levels (between group median change -24.7% ; $P = 0.018$) and fasting apoB48 serum levels (-35.9% ; $P = 0.039$) compared with placebo. Alirocumab reduced the plasma TG area under the curve (AUC) (-26.4% ; $P = 0.006$) and apoB48 AUC (-55.7% ; $P = 0.046$), as well as plasma TG incremental AUC (-21.4% ; $P = 0.04$) and apoB48 incremental AUC

(−26.8%; $P = 0.02$). In addition, alirocumab reduced fasting and postprandial TG levels in very low-density lipoprotein (VLDL) and LDL. Alirocumab improved fasting pulse wave velocity, but no changes in postprandial markers of inflammation were observed.

Conclusions: In addition to the well-known LDL-C-reducing effects, 6 weeks of alirocumab treatment lowered both fasting and postprandial plasma TG levels by reducing the TG levels in VLDL and LDL and the concentration of intestinal remnants.

KEYWORDS

apolipoprotein, lipids and lipoproteins, PCSK9, postprandial, triglycerides

1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major global health problem associated with an increased risk of cardiovascular disease (CVD).^{1,2} Despite advances in the treatment of diabetes and interventions aimed at reducing CVD risk factors,^{3,4} patients with T2DM still have an unacceptable residual CVD risk compared with the general population.⁵ One of the metabolic complications in diabetes is a cluster of atherogenic lipid abnormalities, characterized by elevated levels of plasma triglycerides (TG), reduced concentrations of high-density lipoprotein-cholesterol (HDL-C), increased small dense low-density lipoprotein-cholesterol (LDL-C) and postprandial hyperlipidaemia, including accumulation of atherogenic chylomicron remnants.^{6,7}

Postprandial hyperlipidaemia has been associated with an increased risk of CVD.^{8–10} In addition, evidence for a connection with a systemic inflammatory response by activation of circulating leucocytes and the generation of cytokines has been reported.^{11–14} Furthermore, this postprandial hyperlipidaemia also has been associated with impaired vascular function.^{13,15,16} Improving postprandial hyperlipidaemia may be an important tool in reducing the residual CVD risk present in patients with T2DM.^{17,18}

Statins are the preferred lipid-lowering therapy in T2DM. These drugs preferentially reduce LDL-C, but they also have a mild effect on postprandial levels of TG and apolipoprotein (apo) B48, the structural protein of intestinally derived chylomicrons and their remnants.^{19–23} Recently, a novel treatment has been introduced with proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Inhibition of PCSK9 with antibodies, of which alirocumab and evolocumab are currently used in clinical practice, decreases PCSK9-mediated breakdown of the LDL receptor (LDLR) and consequently increases hepatic LDL uptake via apoB100 recognition,²⁴ leading to lower levels of LDL-C in plasma. Higher LDLR expression may also improve chylomicron and VLDL remnant clearance via the apoE binding domain of the LDLR.²⁵ ApoB48 lacks the carboxy terminal part of the apoB100 molecule, which is the binding domain for the LDLR. Moreover, apoE binding to the LDLR has a higher affinity than apoB binding.²⁵

PCSK9 inhibitors have been shown to reduce CVD risk in various populations, including subgroups of patients with diabetes.^{26,27}

Furthermore, clinical trials have shown that PCSK9 inhibition improves diabetic dyslipidaemia, by reducing LDL-C and increasing HDL-C.²⁸ However, the effects on TG and apoB48 have been less clear, with the majority of studies showing only a modest effect on fasting levels.^{26,27,29} Two previous studies with PCSK9 inhibitors in healthy, normolipidaemic subjects did not show effects on postprandial TG and apoB48 levels.^{20,29} In contrast, patients with loss of function of PCSK9 display postprandial reductions of TG and apoB48 levels.³⁰ To date, the postprandial effects of PCSK9 inhibition in patients with T2DM are unclear.

The present study was designed as an explorative proof-of-concept study to investigate the effects of PCSK9 inhibition with alirocumab on postprandial lipaemia, endothelial function and systemic inflammation in patients with T2DM.

2 | MATERIALS AND METHODS

2.1 | Study design and subjects

This study was designed as a single-centre 1:1 randomized, double-blind, placebo-controlled explorative proof-of-concept study that aimed to compare the effect of alirocumab versus placebo on postprandial lipaemia, endothelial function and inflammation. The study was approved by the Institutional Review Board of the Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands, the regional independent medical research ethics committee TWOR, Rotterdam, and by the national competent authority. All patients provided written informed consent before any specific study procedure. The study was registered at www.trialregister.nl under clinical trial number NTR6709.

Between December 2017 and October 2018, male patients with T2DM on intensive insulin treatment who visited the Diabetes and Vascular Center of the Franciscus Gasthuis & Vlietland (Rotterdam, the Netherlands) were recruited. All consecutive patients with the required characteristics were screened. Inclusion criteria were: male sex, ≥ 18 years old, diagnosis of T2DM, intensive insulin therapy (once-daily long-acting basal insulin and three times daily short-acting insulin, with a stable dosage for 10 weeks) and fasting plasma TG

concentrations between 1.5 and 7.0 mmol/L. Exclusion criteria were: smoking, decreased kidney function (estimated glomerular filtration rate <40 mL/min/1.73 m²), a cardiovascular event, or a severe hypoglycaemic event in the previous 6 months.

2.2 | Data collection

All subjects visited the hospital after a 10-h overnight fast. Anthropomorphic characteristics (length, body weight, body mass index, waist circumference) and blood pressure were measured. The medical and family histories were recorded. A fasting venous blood sample was obtained and baseline arterial stiffness was determined after 5 min of rest. Participants received an oral fat load consisting of fresh cream (Albert Heijn, Zaandam, the Netherlands) in a dose of 50 g of fat per m² body surface.^{31,32} The cream contains a 40% (w/v) fat emulsion with a polyunsaturated/saturated fat ratio of 0.10, containing 0.001% (w/v) cholesterol and 3% (w/v) carbohydrates, representing a total energy content of 3700 kcal/L. During the oral fat loading test, participants were not allowed to eat or to drink (except water) and they were asked to refrain from physical activity. Systolic and diastolic blood pressure, arterial stiffness measurements and venous blood sampling were repeated every 2 h until 8 h after ingestion of the cream.

2.3 | Outcome measures

The primary endpoint was the change in postprandial TG and serum apoB48 levels because of 6 weeks of treatment with alirocumab compared with placebo. Secondary endpoints were the effect of alirocumab on postprandial leucocyte activation markers CD11b, CD66b and CD35, total plasma apoB levels and vascular function.

2.4 | Randomization, blinding and treatment

After the first oral fat loading test, patients were randomized 1:1 to receive subcutaneous injections of either alirocumab 150 mg or matching placebo (both kindly provided by Regeneron) every 2 weeks for 6 weeks. Randomization was based on computer generated block randomization. Both physician and participants were blinded to the allocated strategy. Two weeks after the third injection (6 weeks after the first oral fat load) patients visited the outpatient clinic for the second oral fat loading test. Information on the occurrence and type of side effects of the treatment with either alirocumab or placebo was recorded.

2.5 | Vascular function

Carotid to femoral pulse wave velocity (PWV) and augmentation index (Aix) were measured and calculated with the SphygmoCor Electronics Module MM3 and SphygmoCor CvMS Software Suite version 8.0

(AtCor Medical, West Ryde, New South Wales, Australia). The PWV, which increases at higher aortic stiffness, was measured using a non-invasive tonometry at the carotid and femoral artery. The distance between the carotid and femoral artery was measured with a measuring tape. The Aix, adjusted to a heart rate of 75 beats/min, represents both macrovascular and microvascular function. The radial artery was used to measure the Aix. Both the PWV and the Aix were measured in duplicate at each time interval and the mean value is reported.

2.6 | Laboratory measurements

Most clinical chemistry and haematology measurements were carried out on plasma from freshly drawn blood at the Department of Clinical Chemistry, Franciscus Gasthuis & Vlietland (Rotterdam, the Netherlands) according to standard procedures. C-reactive protein, glucose, total cholesterol, HDL-C and TG were measured using an Architect c8000 (Abbott, Chicago, Illinois). LDL-C values were calculated using the Friedewald formula for TG <4.00 mmol/L. As postprandial TG concentrations rose above 4.00 mmol/L, no postprandial LDL-C was calculated. Plasma apoA-I and apoB (which includes both apoB100 and apoB48) were determined by nephelometry using an IMAGE instrument with commercially available kits (Beckman Coulter, Miami,

TABLE 1 Baseline characteristics of study participants (n = 12)

	Total (n = 12)	Alirocumab (n = 6)	Placebo (n = 6)
Age (years)	62.6 ± 7.8	61.5 ± 9.9	63.7 ± 5.6
Body mass index (kg/m ²)	32.8 ± 5.6	31.5 ± 6.0	34.1 ± 5.5
Waist circumference (cm)	110 ± 21	107 ± 25	113 ± 19
Systolic blood pressure (mmHg)	134 ± 11	133 ± 12	135 ± 10
Fasting glucose (mmol/L)	8.5 ± 1.9	8.3 ± 2.3	8.6 ± 1.7
Glycated A1c (mmol/mol)	65.6 ± 8.7	63.5 ± 7.7	65.5 ± 10.9
Total cholesterol (mmol/L)	4.0 ± 0.8	3.8 ± 0.7	4.2 ± 0.9
HDL-C (mmol/L)	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.1
LDL-C (mmol/L)	1.9 ± 0.5	1.9 ± 0.5	2.0 ± 0.5
Plasma TG (mmol/L)	1.89 (0.92–2.87)	1.75 (0.86–2.59)	2.64 (1.69–3.59)
ApoA1 (g/L)	1.24 ± 0.12	1.23 ± 0.10	1.25 ± 0.14
ApoB (g/L)	0.85 ± 0.20	0.82 ± 0.15	0.89 ± 0.25
ApoB48 (mg/L)	12.9 (4.4–21.4)	9.3 (0.0–19.2)	18.8 (8.2–29.4)
Lipoprotein(a) (mg/L)	120.5 (0.0–376.0)	63.0 (0.0–414.5)	115.0 (0.0–255.5)

Abbreviations: Apo, apolipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Data as mean ± SD or median (interquartile range). There were no significant differences between treatment groups.

TABLE 2 Changes in fasting plasma parameters before and after intervention

	Alirocumab (n = 6)			Placebo (n = 6)			Between group difference	
	Baseline	Week 6	% Change from baseline	Baseline	Week 6	% Change from baseline	Median % change from baseline	P-model
Total cholesterol (mmol/L)	3.8 ± 0.7	2.4 ± 0.2*	-46.2	4.2 ± 0.9	3.8 ± 0.6	-9.5	-28.2	<0.001
HDL-C (mmol/L)	1.0 ± 0.2	1.1 ± 0.2	+10.0	1.0 ± 0.1	0.9 ± 0.1	-10.0	+5.9	0.18
LDL-C (mmol/L)	1.9 ± 0.5	0.7 ± 0.2*	-63.1	2.0 ± 0.5	1.7 ± 0.4	-15.0	-39.5	<0.001
Plasma TG (mmol/L)	1.74 (1.01–2.47)	1.41 (0.97–1.95)*	-19.0	2.64 (1.69–3.59)	2.96 (2.08–3.84)	+12.1	-24.7	0.006
ApoA1 (g/L)	1.23 ± 0.10	1.31 ± 0.22	+6.5	1.25 ± 0.14	1.20 ± 0.18	-4.0	+8.0	0.27
ApoB (g/L)	0.82 ± 0.15	0.41 ± 0.09*	-50.0	0.85 ± 0.25	0.86 ± 0.16	+1.2	-50.7	<0.001
ApoB48 (mg/L)	9.3 (2.0–16.7)	7.0 (4.9–9.2)	-24.5	18.9 (3.2–34.4)	21.3 (5.1–37.4)	+12.7	-35.9	0.039
uLDL-TG (mmol/L)	0.19 ± 0.08	0.12 ± 0.02*	-36.2	0.19 ± 0.06	0.19 ± 0.05	0.0	-25.6	0.003
uVLDL-TG (mmol/L)	0.69 ± 0.24	0.52 ± 0.25*	-24.4	1.08 ± 0.34	1.06 ± 0.59	-1.1	-29.1	0.043
uHDL-C (mmol/L)	0.79 ± 0.32	0.83 ± 0.30	+5.1	0.71 ± 0.12	0.69 ± 0.13	-2.3	+8.3	0.23
uLDL-C (mmol/L)	1.21 ± 0.23	0.56 ± 0.16*	-53.7	1.28 ± 0.30	1.21 ± 0.28	-5.5	-39.9	<0.001
uVLDL-C (mmol/L)	0.71 ± 0.25	0.41 ± 0.15*	-42.3	0.65 ± 0.14	0.70 ± 0.26	+7.7	-41.2	<0.001
uLDL-apoB (mmol/L)	0.40 ± 0.06	0.18 ± 0.06*	-55.0	0.45 ± 0.09	0.44 ± 0.12	-2.3	-44.1	<0.001
uVLDL-apoB (mmol/L)	0.09 ± 0.02	0.05 ± 0.02*	-44.4	0.13 ± 0.05	0.14 ± 0.04	+7.7	-65.7	<0.001
Plasma free fatty acids (mmol/L)	0.55 ± 0.23	0.41 ± 0.16	-25.5	0.61 ± 0.19	0.63 ± 0.28	+3.3	-21.4	0.15
Plasma glucose (mmol/L)	8.3 ± 1.9	8.7 ± 2.0	+4.8	8.6 ± 1.7	8.3 ± 1.9	-3.3	+6.8	0.55
HbA1c (mmol/mol)	63.5 ± 7.7	60.0 ± 4.1	-5.5	65.5 ± 10.9	64.7 ± 10.1	-1.1	-0.4	0.14

Abbreviations: Apo, apolipoprotein; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; VLDL-C, very low-density lipoprotein cholesterol. u depicts measurements after serum ultracentrifugation.

Data as mean ± SD or median (interquartile range). P-model by ANCOVA with post-treatment levels as dependent variable and pre-treatment levels as covariate and treatment group as fixed effect. *P < 0.05 versus baseline.

Florida). Blood cell counts and five-part leucocyte differentiation were determined using LH750 or DxH analysers (Beckman Coulter). Haemoglobin A1c (HbA1c) was measured with a G8 analyser (Tosoh, San Francisco, California).

ApoB48 serum concentrations were quantified using a commercially available enzyme-linked immunoassay (ELISA) (Shibayagi Co., Ltd., Shibukawa, Japan).³³ As no commercial quality controls are available for apoB48, a local internal quality control was pooled according to WHO recommendations, stored at -80°C and then assayed in duplicate on each plate, in parallel to samples. Paired samples were measured on the same run.

Free fatty acids (FFAs) were measured by an enzymatic colorimetric assay (WAKO kit, Fujifilm WAKO Diagnostics, Richmond, Virginia) at the Division of Endocrinology, Leiden University Medical Center (Leiden, the Netherlands). Soluble intracellular molecule 1 concentrations were determined using commercially available ELISA kits (Invitrogen, Carlsbad, California). Interleukin 1β concentrations were determined using a commercially available ELISA kit (R&D Systems, Minneapolis, Minnesota).

Leucocyte activation markers were measured in EDTA blood as described in detail before.^{11,12} Briefly, the staining procedure was started within 30 min after venepuncture. All measurements were

carried out in triplicate and the mean value is reported. Whole blood was added to a combination of fluorescein isothiocyanate (FITC)-conjugated CD66b, phycoerythrin cyanin (PC5)-conjugated CD11b, phycoerythrin (PE)-conjugated CD35 and phycoerythrin-Texas Red-X-conjugated CD45. In parallel, blood was added to a combination of FITC, and PC5- and PE-conjugated mouse IgG1 as isotype controls to correct for non-specific binding. All antibodies were from Beckman Coulter, except for CD35-PE (BD Bioscience, Franklin Lakes, New Jersey). Cells were incubated for 15 min in the dark at room temperature. Erythrocytes were subsequently lysed by adding isotonic erythrocyte lysing solution (in mol/L: 0.19 ammonium chloride, 0.01 potassium hydrogen carbonate, 0.12 EDTA, pH 7.2) for 15 min. An FC500 flow cytometer and Kaluza 1.5a (Beckman Coulter) were used for measurement and analysis. Lymphocytes, monocytes and neutrophil granulocytes were identified based on their side scatter and the level of CD45 on their surface. The fluorescent intensity of each cell population was expressed as the mean fluorescent intensity in arbitrary units.

Lipoprotein profiles were obtained using density gradient ultracentrifugation³⁴ for time-points $t = 0$, $t = 4$ and $t = 8$ h. KBr was added to plasma (0.35 g/mL) to obtain a density of 1.26 g/mL. Of this plasma, 1 mL was placed in an ultracentrifuge tube and 19 mL of KBr solutions

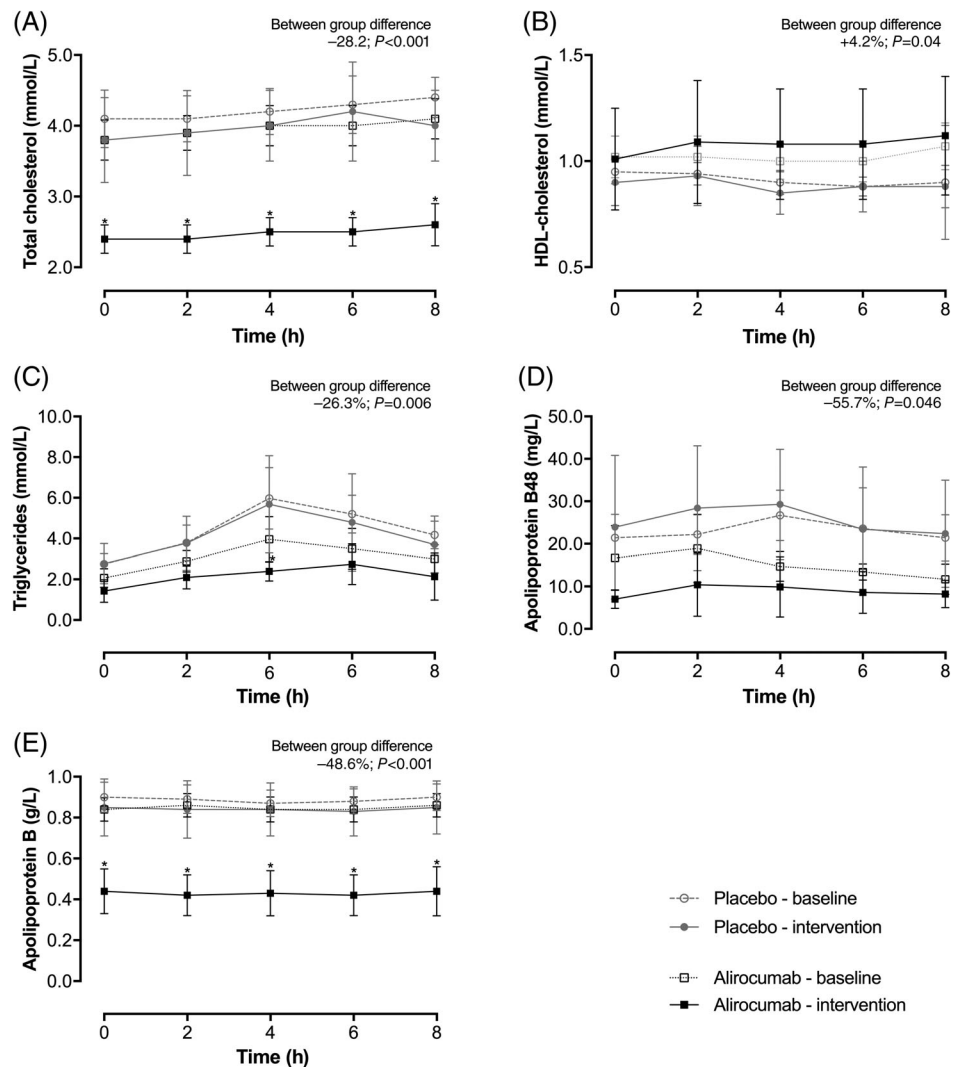


FIGURE 1 Total cholesterol, HDL-cholesterol, triglycerides, apoB48, and total apoB concentrations after an oral fat load test. Mean \pm SEM of total cholesterol (A), HDL-cholesterol (B), triglycerides (C), apoB48 (D) and apoB (E) at baseline (open square) and after treatment (closed square) with alirocumab and at baseline (open circle) and after treatment (closed circle) with placebo during an oral fat load test. $*P < 0.05$ versus placebo for treatment with alirocumab. There were no significant differences at baseline or after treatment for the placebo group. Apo, apolipoprotein; HDL, high-density lipoprotein

of 1.21, 1.10, 1.063, 1.04 and 1.02 g/mL in physiological salt were layered successively on top, followed by 1 mL of water. Samples were centrifuged at 207 000 g for 18 h at 4°C using a SW41 rotor in a L-70 Beckman ultracentrifuge (Beckman Instruments, Indianapolis, Indiana). After each run, the density gradient was fractionated from the bottom into 250 µL fractions. Lipoproteins were separated based on density. VLDL, chylomicrons and large remnants were recovered in the 1.02 g/mL range; this fraction is designated uVLDL. LDL was recovered in fractions with densities ranging from 1.04 to 1.063 g/mL; this fraction is designated uLDL. Fractions with densities ranging from 1.063 to 1.21 g/mL were considered to constitute HDL and designated uHDL. (NB: 'u' depicts measurements after serum ultracentrifugation.)

Cholesterol, TG and apoB in the fractions were measured using a Selectra E (DDS Diagnostic system, Istanbul, Turkey).

2.7 | Statistical analysis

Data are given as mean ± SD for normally distributed variables, median (interquartile range) for variables with skewed distribution

(plasma TG, apoB48, C-reactive protein and PWV) and number (%) for categorical variables. In all figures, data are presented as mean ± SEM.

The main treatment effect (*P*-model) of alirocumab versus placebo on outcome values between pre- and post-treatment levels was tested with ANCOVA with the post-treatment concentrations as the dependent variable, pretreatment concentrations as covariate and treatment group as fixed effect. Data were presented as median change in percentage of outcome value compared with baseline by alirocumab versus placebo.

Differences within groups before and after treatment (for both fasting and postprandial concentrations) were determined by the paired *t*-test or Wilcoxon matched pair signed rank test, depending on the skewness of the distribution. Differences between groups were determined by the independent Student's *t*-test or Mann-Whitney *U*-test, where appropriate. All postprandial responses were calculated by the area under the curve (AUC), using the trapezoidal rule with GraphPad Prism version 5.0 (Los Angeles, California). Incremental AUC (iAUC) was calculated after correction for fasting concentrations.

TABLE 3 Changes (area under the curve) in postprandial lipid and cholesterol levels

	Alirocumab (n = 6)			Placebo (n = 6)			Between group difference	
	Baseline	Week 6	% Change from baseline	Baseline	Week 6	% Change from baseline	Median % change from baseline	<i>P</i> -model
Total cholesterol								
AUC (mmol/L 8 h)	31.5 ± 5.7	19.8 ± 1.7*	-37.1	34.2 ± 6.5	32.0 ± 4.7	-6.4	-28.2	<0.001
iAUC (mmol/L 8 h)	1.5 ± 0.6	0.9 ± 0.6*	-38.4	1.1 ± 0.9	1.3 ± 1.1	+14.2	-19.2	0.01
HDL-C								
AUC (mmol/L 8 h)	8.1 ± 1.9	8.7 ± 2.1	+7.4	7.4 ± 1.0	7.1 ± 0.9	-4.1	+4.2	0.04
iAUC (mmol/L 8 h)	0.3 ± 0.3	0.6 ± 0.2*	+97.6	0.3 ± 0.2	0.3 ± 0.3	+1.4	+23.2	0.03
Total TG								
AUC (mmol/L 8 h)	25.8 (16.4-35.2)	18.0 (14.5-21.5)	-30.2	36.8 (21.8-51.8)	35.0 (23.8-46.2)	-4.9	-26.3	0.01
iAUC (mmol/L 8 h)	8.6 (3.2-11.8)	6.2 (4.6-7.8)	-29.4	11.8 (4.1-19.4)	12.4 (6.1-18.7)	+0.8	-21.4	0.04
ApoB								
AUC (g/L 8 h)	6.8 ± 1.1	3.4 ± 0.8*	-50.1	7.1 ± 1.3	6.8 ± 1.0	-4.3	-48.6	<0.001
iAUC (g/L 8 h)	0.2 ± 0.2	0.1 ± 0.2	-39.3	0.2 ± 0.1	0.2 ± 0.2	-2.5	-28.3	0.04
ApoB48								
AUC (mg/L 8 h)	105.6 (43.5-167.7)	69.2 (38.3-100.1)*	-34.5	207.5 (147.5-267.5)	201.8 (83.1-320.5)	-3.7	-55.7	0.046
iAUC (mg/L 8 h)	15.8 (0.0-33.5)	9.7 (4.0-15.4)	-28.8	27.0 (0.0-63.0)	30.5 (22.2-38.8)	+4.7	-26.8	0.02
Free fatty acids								
AUC (mmol/L 8 h)	6.5 ± 2.4	5.9 ± 1.9	-9.2	6.7 ± 1.1	6.7 ± 2.4	-0.9	-9.8	0.34
iAUC (mmol/L 8 h)	0.7 ± 1.3	0.8 ± 0.9	+1.3	0.8 ± 0.8	0.8 ± 1.0	+2.3	+1.3	0.53

Abbreviations: Apo, apolipoprotein; AUC, area under the curve; HDL, high-density lipoprotein; iAUC, incremental area under the curve.

Data as mean ± SD or median (interquartile range). *P*-model by ANCOVA with post-treatment levels as dependent variable and pretreatment levels as covariable.

**P* <0.05 versus baseline.

Statistical analysis was carried out with PASW statistics version 25.0 (IBM SPSS Statistics, New York). $P < 0.05$ (two-tailed) was considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics

Twelve eligible patients were randomized to either alirocumab ($n = 6$) or placebo ($n = 6$). The mean age was 62.6 ± 7.8 years, all were on statin treatment with a mean plasma LDL-C of 1.9 ± 0.5 mmol/L, and median plasma TG concentrations of 1.89 (0.93–2.87) mmol/L. Additional baseline characteristics are shown in Table 1. No serious adverse events were recorded.

3.2 | Effect of alirocumab on fasting lipid concentrations

Table 2 shows the fasting and postprandial lipid concentrations before and after treatment. Six weeks of alirocumab treatment significantly decreased fasting concentrations of total cholesterol (median change of -28.2% ; P -model < 0.001), LDL-C (-39.5% ; $P < 0.001$), plasma TG (-24.7% ; $P = 0.02$), plasma apoB (-50.7% ; $P = < 0.001$) and apoB48 (-35.9% ; $P = 0.04$) compared with placebo. Alirocumab did not significantly affect HbA1c, FFA or glucose concentrations.

3.3 | Effects of alirocumab on postprandial plasma triglyceride and apolipoprotein B48 concentrations

Alirocumab resulted in a significantly reduced plasma TG-AUC (median change of -26.3% ; P -model 0.006) and apoB48-AUC response (-55.7% ; $P = 0.046$) compared with placebo (Figure 1 and Table 3). In addition, reductions in plasma TG-iAUC (-21.4% ; $P = 0.04$) and apoB48-iAUC (-26.8% ; $P = 0.02$) compared with placebo were found. Furthermore, total cholesterol-AUC (-28.2% ; $P < 0.001$), plasma apoB-AUC (-48.6% ; $P < 0.001$) and HDL-C-AUC ($+4.2\%$; $P = 0.04$) changed after alirocumab, while FFA remained unaffected.

3.4 | Effects of alirocumab on lipoprotein profiles

Compared with placebo, alirocumab reduced fasting uVLDL-TG (median change -29.1% ; P -model 0.04), uVLDL-C (-41.2% , $P < 0.001$) and uVLDL-apoB (-65.7% ; $P < 0.001$). Reductions were also observed for fasting uLDL-TG (-25.6% ; $P = 0.003$), uLDL-C (-39.9% ; $P < 0.001$) and uLDL-apoB (-44.1% ; $P < 0.001$) (Table 2 and Figure S1; see Supporting Information).

In addition, after 4 and 8 h, significant reductions of uVLDL-TG, uVLDL-C, uVLDL-apoB and uLDL-TG, uLDL-C and uLDL-apoB were observed (see Table S1; see Supporting Information).

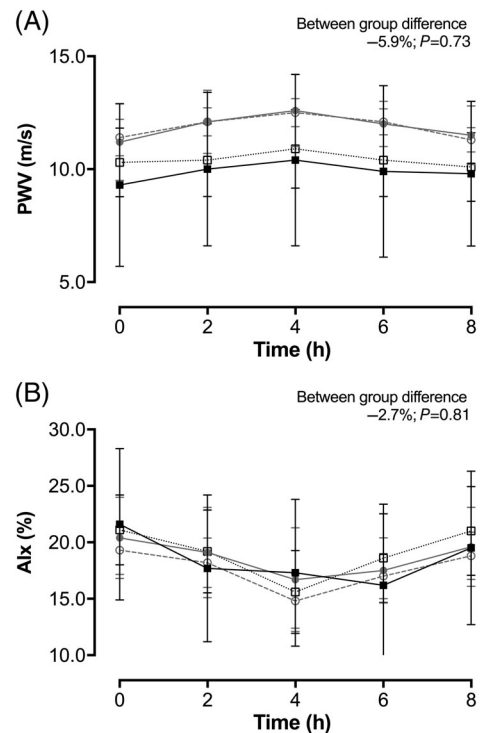


FIGURE 2 PWV and pulse wave Aix after an oral fat load test. Mean \pm SEM of PWV (A) and pulse wave Aix (B) at baseline (open square) and after treatment (closed square) with alirocumab and at baseline (open circle) and after treatment (closed circle) with placebo during an oral fat load test. No significant postprandial differences were observed between groups at baseline or after treatment. Aix, augmentation index; PWV, pulse wave velocity

3.5 | Effects of alirocumab on inflammatory parameters and vascular function

Alirocumab did not significantly affect fasting or postprandial leucocyte counts or leucocyte activation markers (see Tables S2 and S3, Figure S2; see Supporting Information).

Fasting PWV was reduced by alirocumab compared with placebo (-7.9% ; $P = 0.03$); however, no significant differences in postprandial changes of PWV were observed (Figure 2 and Table S3; see Supporting Information). No significant changes were observed in Aix.

4 | DISCUSSION

In this proof-of-concept study, we investigated the effects of alirocumab on postprandial concentrations of lipids and lipoproteins and on postprandial inflammation in male patients with T2DM on intensive insulin treatment. Alirocumab significantly reduced fasting plasma TG and apoB48 concentrations as well as the postprandial plasma response of TG and apoB48. Furthermore, significant reductions in the TG, cholesterol and apoB fractions of uLDL and uVLDL were observed.

Two previous studies in healthy subjects did not find changes in fasting TG or postprandial plasma TG responses by PCSK9 inhibition.^{20,29} The most likely explanation for the different finding is the fact that our study included only patients with T2DM. Patients with diabetes are characterized by an increased production and an impaired clearance of both chylomicrons and VLDL,³⁵ leading to a state of competition in the clearance of these particles. The improved clearance of these TG-rich lipoproteins (TRL) by PCSK9 inhibition might only be revealed when the system is “under pressure”, such as in patients with diabetes. To our knowledge, this is the first study investigating the effect of PCSK9 on postprandial lipid metabolism in patients with T2DM.

We propose that the reduction in fasting and postprandial plasma TG as observed here is partly caused by increased catabolic efficacy because of reduced competition at the level of the lipolytic enzymes involved in the clearance of TRL [lipoprotein lipase (LPL) and hepatic lipase]. As the majority of TGs reside in chylomicrons and VLDL, the strong reduction of these TRL resulted in reduced levels of plasma TGs.

PCSK9 inhibition leads to upregulation of the hepatic LDLR, which besides LDL particles also recognizes and binds apoE-containing particles, including TRL remnants.²⁵ Therefore, upregulation of the LDLR leads to increased removal of LDL and enhanced direct uptake of remnants (both chylomicron and VLDL remnants). Chylomicrons normally compete with VLDL for the same lipolytic pathways, leading to accumulation of endogenous VLDL in the postprandial state.^{36,37} An increased TRL clearance should therefore result in increased LPL-mediated lipolysis because of lower competition. In the present study, we found decreased fasting and postprandial (at $t = 4$ h) concentrations of uVLDL-TG and uVLDL-C. This is in line with a report by Chan et al, who previously described that PCSK9 inhibition leads to decreased postprandial VLDL-apoB100.²⁰

In addition, there is evidence showing that PCSK9 inhibition is not only involved in the LDLR expression, but also leads to reduced degradation of the VLDL receptor.^{38,39} The VLDL receptor plays an important role in postprandial lipoprotein metabolism by enhancing the LPL-mediated TG hydrolysis,⁴⁰ which could enhance the TG-lowering effects of alirocumab.

The reduction of postprandial lipaemia by PCSK9 inhibition, as shown in this study, could be an important factor in the reduction of the excess CVD risk in patients with T2DM;⁴¹ in particular, because there is an abundance of evidence that the non-fasting TGs, which are related to chylomicron remnants, are associated with higher risk of CVD compared with fasting LDL-C.⁹ In addition, it has been shown that chylomicron remnants have a longer retention time in the arterial wall compared with LDL.⁴² As arterial wall retention of lipoproteins results in atherosclerosis,⁴³ reductions of these postprandial lipoproteins could be beneficial in terms of cardiovascular risk.

Reductions in postprandial concentrations of apoB48 have been found in studies evaluating the postprandial lipoprotein metabolism with HMG-CoA reductase inhibitors (statins) in patients with diabetes. For example, Battula et al showed that cerivastatin reduced TGs and cholesterol in the TRL fractions of patients with diabetes.²¹

Besides the effects on lipaemia and inflammation, we also explored the effects of PCSK9 inhibition on vascular function. It has been shown that an oral fat load acutely impairs vascular function.^{13,15,16} Furthermore, several trials have shown that an intervention with statins improves vascular function^{44,45} and we have described an effect on postprandial leucocyte activation.⁴⁶ Our group has published repeatedly on this postprandial inflammation and the effects on postprandial vascular function.^{47,48} In the present trial only a reduction in fasting PWV was found and no postprandial changes were observed. This can mainly be explained by the fact that our study group was small and the variance in measurements of both PWV and Aix was relatively large, particularly in the postprandial period. This may also explain the lack of effects of inflammatory parameters.

Importantly, we observed a non-significant trend for higher fasting TG and apoB48 concentrations in the placebo group versus the alirocumab group. This could have affected our results, as fasting plasma TG levels are an important determinant of the postprandial TG response.^{49,50} To address this issue, we corrected for baseline levels in our main comparison between groups and used postprandial responses for all variables. The incremental data are in line with the unadjusted results.

In conclusion, this study suggests that in male patients with T2DM who are on an intensive insulin regimen, inhibition of PCSK9 with alirocumab resulted in a decreased postprandial plasma TG response because of lower chylomicrons and their remnants, but also in a decrease in other lipoprotein fractions. This mechanism may contribute to the observed improved vascular function described here and the studies showing reductions in cardiovascular events in patients with T2DM on PCSK9 inhibitors, in addition to the potent effects on LDL-C.

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CONFLICT OF INTEREST

M.C.C. served as a member of different advisory boards and received fees for educational lectures from Sanofi and Amgen. The other authors report no conflict of interest.

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REFERENCES

1. Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: insights from mechanistic studies. *Lancet*. 2008; 371(9626):1800-1809.
2. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA*. 2002;287(19): 2570-2581.
3. Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V, Patel A. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. *JAMA*. 2015;313(6):603-615.

4. Cholesterol Treatment Trialists' (CTT) Collaborators, Kearney PM, Blackwell L, Collins R, et al. Efficacy of cholesterol-lowering therapy in 18 686 people with diabetes in 14 randomised trials of statins: a meta-analysis. *Lancet*. 2008;371(9607):117-125.
5. Rawshani A, Rawshani A, Franzén S, et al. Mortality and cardiovascular disease in type 1 and type 2 diabetes. *N Engl J Med*. 2017;376(15):1407-1418.
6. Taskinen M-R. Diabetic dyslipidaemia: from basic research to clinical practice. *Diabetologia*. 2003;46(6):733-749.
7. Ginsberg HN, Illingworth DR. Postprandial dyslipidemia: an atherogenic disorder common in patients with diabetes mellitus. *Am J Cardiol*. 2001;88(6):9-15.
8. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation*. 2008;118(10):993-1001.
9. 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.
10. Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis*. 1994;106(1):83-97.
11. Alipour A, Van Oostrom AJ, Izraeljan A, et al. Leukocyte activation by triglyceride-rich lipoproteins. *Arterioscler Thromb Vasc Biol*. 2008;28(4):792-797.
12. van Oostrom AJ, Rabelink TJ, Verseyden C, et al. Activation of leukocytes by postprandial lipemia in healthy volunteers. *Atherosclerosis*. 2004;177(1):175-182.
13. van Oostrom AJ, Sijmonsma TP, Verseyden C, et al. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J Lipid Res*. 2003;44(3):576-583.
14. Gower RM, Wu H, Foster GA, et al. CD11c/CD18 Expression is upregulated on blood monocytes during hypertriglyceridemia and enhances adhesion to vascular cell adhesion molecule-1. *Arterioscler Thromb Vasc Biol*. 2011;31(1):160-166.
15. Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am J Cardiol*. 1997;79(3):350-354.
16. Marchesi S, Lupattelli G, Schillaci G, et al. Impaired flow-mediated vasoactivity during post-prandial phase in young healthy men. *Atherosclerosis*. 2000;153(2):397-402.
17. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. *JAMA*. 2007;298(3):309-316.
18. 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.
19. Parhofer KG, Laubach E, Barrett PHR. Effect of atorvastatin on postprandial lipoprotein metabolism in hypertriglyceridemic patients. *J Lipid Res*. 2003;44(6):1192-1198.
20. Chan DC, Watts GF, Somaratne R, Wasserman SM, Scott R, Barrett PHR. Comparative effects of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) inhibition and statins on postprandial triglyceride-rich lipoprotein metabolism. *Arterioscler Thromb Vasc Biol*. 2018;38(7):1644-1655.
21. Battula SB, Fitzsimons O, Moreno S, et al. Postprandial apolipoprotein B48- and B100-containing lipoproteins in type 2 diabetes: do statins have a specific effect on triglyceride metabolism? *Metabolism*. 2000;49(8):1049-1054.
22. Otokoza S, Ai M, Van Himbergen T, et al. Effects of intensive atorvastatin and rosuvastatin treatment on apolipoprotein B-48 and remnant lipoprotein cholesterol levels. *Atherosclerosis*. 2009;205(1):197-201.
23. Ng TWK, Watts GF, Stuckey BGA, et al. Does pravastatin increase chylomicron remnant catabolism in postmenopausal women with type 2 diabetes mellitus? *Clin Endocrinol (Oxf)*. 2005;63(6):650-656.
24. Costet P, Krempf M, Cariou B. PCSK9 and LDL cholesterol: unravelling the target to design the bullet. *Trends Biochem Sci*. 2008;33(9):426-434.
25. Mahley RW, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J Lipid Res*. 1999;40(1):1-16.
26. Schwartz GG, Steg PG, Szarek M, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med*. 2018;379(22):2097-2107.
27. Sabatine MS, Leiter LA, Wiviott SD, et al. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. *Lancet Diabetes Endocrinol*. 2017;5(12):941-950.
28. Seiler C, Pennec X, Reyes M. Capturing the multiscale anatomical shape variability with polyaffine transformation trees. *Med Image Anal*. 2012;16(7):1371-1384.
29. Reyes-Soffer G, Pavlyha M, Ngai C, et al. Effects of PCSK9 inhibition with alirocumab on lipoprotein metabolism in healthy humans clinical perspective. *Circulation*. 2017;135(4):352-362.
30. Ooi TC, Krysa JA, Chaker S, et al. The effect of PCSK9 loss-of-function variants on the postprandial lipid and ApoB-lipoprotein response. *J Clin Endocrinol Metab*. 2017;102(9):3452-3460.
31. De Vries MA, Van Der Meulen N, Van De Geijn GJM, et al. Effect of a single dose of Vitamin D3 on postprandial arterial stiffness and inflammation in Vitamin D-deficient women. *J Clin Endocrinol Metab*. 2017;102(3):992-1000.
32. Klop B, van de Geijn G-JM, Birnie E, et al. Vitamin D3 mediated effects on postprandial leukocyte activation and arterial stiffness in men and women. *Eur J Clin Nutr*. 2014;68(5):635-637.
33. Kinoshita M, Kojima M, Matsushima T, Teramoto T. Determination of apolipoprotein B-48 in serum by a sandwich ELISA. *Clin Chim Acta*. 2005;351(1-2):115-120.
34. Versmissen J, Vongpromek R, Yahya R, et al. Familial hypercholesterolaemia: cholesterol efflux and coronary disease. *Eur J Clin Invest*. 2016;46(7):643-650.
35. Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? *Diabetologia*. 2015;58(5):886-899.
36. Brunzell JD, Hazzard WR, Porte D, Bierman EL. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J Clin Invest*. 1973;52(7):1578-1585.
37. Bjorkegren J, Packard CJ, Hamsten A, et al. Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J Lipid Res*. 1996;37(1):76-86.
38. Roubtsova A, Munkonda MN, Awan Z, et al. Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue. *Arterioscler Thromb Vasc Biol*. 2011;31(4):785-791.
39. Poirier S, Mayer G, Benjannet S, 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.
40. Goudriaan JR, Santo SMSE, Voshol PJ, et al. The VLDL receptor plays a major role in chylomicron metabolism by enhancing LPL-mediated triglyceride hydrolysis. *J Lipid Res*. 2004;45(8):1475-1481.
41. Nichols GA, Philip S, Reynolds K, Granowitz CB, Fazio S. Increased residual cardiovascular risk in patients with diabetes and high versus normal triglycerides despite statin-controlled LDL cholesterol. *Diabetes Obes Metab*. 2019;21(2):366-371.
42. Proctor SD, Pabla CK, Mamo JCL. Arterial intimal retention of proatherogenic lipoproteins in insulin deficient rabbits and rats. *Atherosclerosis*. 2000;149(2):315-322.

43. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol.* 1995;15(5):551-561.
44. Marchesi S, Lupattelli G, Siepi D, et al. Short-term atorvastatin treatment improves endothelial function in hypercholesterolemic women. *J Cardiovasc Pharmacol.* 2000;36(5):617-621.
45. Nakamura T, Uematsu M, Yoshizaki T, Kobayashi T, Watanabe Y, Kugiyama K. Improvement of endothelial dysfunction is mediated through reduction of remnant lipoprotein after statin therapy in patients with coronary artery disease. *J Cardiol.* 2019; pii: S0914-5087(19)30265-5. <https://doi.org/10.1016/j.jjcc.2019.08.006>.
46. Van Oostrom AJ, Plokker HW, Van Asbeck BS, et al. Effects of rosuvastatin on postprandial leukocytes in mildly hyperlipidemic patients with premature coronary sclerosis. *Atherosclerosis.* 2006;185(2):331-339.
47. de Vries MA, Klop B, Janssen HW, Njo TL, Westerman EM, Castro CM. Postprandial inflammation: targeting glucose and lipids. *Adv Exp Med Biol.* 2014;824:161-170.
48. Klop B, Proctor SD, Mamo JC, Botham KM, Castro Cabezas M. Understanding postprandial inflammation and its relationship to lifestyle behaviour and metabolic diseases. *Int J Vasc Med.* 2012;2012: 947417.
49. Lewis GF, O'Meara NM, Soltys PA, et al. Fasting hypertriglyceridemia in noninsulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. *J Clin Endocrinol Metab.* 1991;72(4):934-944.
50. Christen T, de Mutsert R, Gast KB, et al. Association of fasting triglyceride concentration and postprandial triglyceride response with the carotid intima-media thickness in the middle aged: The Netherlands Epidemiology of Obesity study. *J Clin Lipidol.* 2017;11(2):377-385.e1.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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