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Liraglutide in combination with metformin may improve the atherogenic lipid profile and decrease C-reactive protein level in statin treated obese patients with coronary artery disease and newly diagnosed type 2 diabetes: A randomized trial

Christian Anholm[a,b,*], Preman Kumarathurai[c], Lene Rørholm Pedersen[c], Amirsalar Samkani[d], Rosemary L. Walzem[e], Olav Wendelboe Nielsen[c], Ole Peter Kristiansen[c], Mogens Fenger[f], Sten Madsbad[g], Ahmad Sajadieh[c], Steen Bendix Haugaard[b,d]

[a] Department of Internal Medicine, Copenhagen University Hospital, Glostrup, Denmark
[b] Department of Internal Medicine, Copenhagen University Hospital, Amager, Denmark
[c] Department of Cardiology, Copenhagen University Hospital, Bispebjerg, Denmark
[d] Department of Endocrinology, Copenhagen University Hospital, Bispebjerg, Denmark
[e] Department of Poultry Science and Faculty of Nutrition, Texas A&M University, Texas, USA
[f] Department of Clinical Biochemistry, Copenhagen University Hospital, Hvidovre, Denmark
[g] Department of Endocrinology, Copenhagen University Hospital, Hvidovre, Denmark

HIGHLIGHTS

- Liraglutide combined with metformin improved the atherogenic LDL lipid profile.
- The most atherogenic subfraction LDL_5 is primarily reduced.
- The combination improved CRP, but had no effect on TNF-α.

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ABSTRACT

Background and aims: Atherosclerosis in obesity and type 2 diabetes (T2DM) is associated with low-grade inflammation (LGI) and dyslipidemia, where especially small, dense lipoprotein particles are atherogenic. The glucagon-like peptide-1 receptor agonist, liraglutide, reduces cardiovascular events by poorly understood mechanisms. We investigated the effect of liraglutide combined with metformin on LGI and lipoprotein density profiles in patients with stable coronary artery disease (CAD) and newly diagnosed T2DM.

Methods: We conducted a randomized, double-blind, placebo-controlled, cross-over trial over a 12 + 12-week period, with ≥2-week wash-out. Intervention: liraglutide/metformin *vs.* placebo/metformin. Lipoproteins were separated by continuous density gradient ultracentrifugation, and LDL divided into five subfractions between 226 and 270 Å, considering particle size ≤255 Å as the atherogenic pattern. Plasma C-reactive protein and tumor necrosis factor-α were assessed by the enzyme-linked immunosorbent-assay.

Results: 28 out of 41 randomized patients completed all visits. Intention-to-treat analysis was performed but one patient had statin dosage and was excluded from the analysis. 95% of the patients were on statin therapy. Overall, liraglutide did not affect lipid subfractions or markers of LGI compared to placebo. The combination of liraglutide and metformin reduced the total LDL subfractions, primarily by reducing the most atherogenic subfraction LDL_5, and reduced CRP but not TNF-α. Explorative analyses suggested that the subfraction LDL_5 during the wash-out period rebounded significantly at least in a per-protocol analysis of the sub-group of patients starting the liraglutide therapy.

Conclusions: In patients with CAD and newly diagnosed T2DM on stable statin therapy, liraglutide combined with metformin may improve the atherogenic LDL lipid profile and CRP.

* Corresponding author. Department of Internal Medicine, Copenhagen University Hospital, Glostrup, Nordre Ringvej 57, 2600, Glostrup, Denmark.
E-mail address: canholm@youmail.dk (C. Anholm).

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1. Introduction

Type 2 diabetes (T2DM) is a major cause of atherosclerosis and an independent risk factor of cardiovascular adverse events [1], and the majority of these patients die from complications of atherosclerosis despite intensive preventive interventions [2]. Insulin resistance and obesity [3], as well as low-grade inflammation [4], contributes to the development of T2DM and atherogenic dyslipidemia. Elevated levels of low-density lipoprotein cholesterol (LDL-C) are a principal risk factors [5], especially when carried in small, dense LDL particles [6], and a reduction hereof is essential to reduce cardiovascular events [7]. Nevertheless, a more accurate risk estimation could be established by analysis of particle size and density, considering the small, dense atherogenic lipoprotein particles with triglyceride-rich, cholesterol-depleted cores [8–11]. Metformin is associated with reduction of cardiovascular morbidity and mortality, as well as improvement in the lipid profile [12]. The glucagon-like peptide-1 receptor agonist (GLP-1RA) liraglutide, used to treat hyperglycemia in T2DM, reduces cardiovascular events [13] by poorly understood mechanisms. Treatment with GLP-1RA is associated with minor reductions in LDL-C [14,15], and liraglutide induces a substantial reduction in postprandial atherogenic remnant particles [16], However, evidence is needed to determine if these improvements might translate into reductions in cardiovascular outcomes. The markers of low-grade inflammation, tumor necrosis factor alfa (TNF-α) and C-reactive protein (CRP), are associated with risk of development of atherosclerosis and risk of developing T2DM in patients with manifest arterial disease [17,18], and predict a poor prognosis in patients with coronary artery disease (CAD) [19,20]. In obese patients with T2DM, liraglutide reduces CRP [21] while results on the effect on TNF-α are conflicting [22–24].

The aim of the present study was to investigate the effect of liraglutide in combination with metformin on established cardiovascular risk factors related to low-grade inflammation and lipoprotein density in patients with CAD and newly diagnosed T2DM.

2. Materials and methods

2.1. Design

The study is an investigator-initiated, double-blind, randomized, placebo-controlled, cross-over trial. Details of the design, population and intervention have been published previously [25].

2.2. Participants

Patients with stable CAD, body mass index (BMI) $\geq 25\ kg/m^2$, age 18–85 years and newly diagnosed (< 2 years) T2DM, according to the criteria defined by the American Diabetes Association [26], were included. Prior to inclusion, patients were treated with diet, metformin or sulfonylurea, alone or in combinations, and statins. All oral antidiabetic medications were ended 2 weeks before the baseline visit. The main exclusion criterion was previous treatment with GLP-1RA or dipeptidyl peptidase-4 inhibitor (DPP-4i). A comprehensive list of the exclusion criteria can be found elsewhere [25].

2.3. Intervention

Liraglutide + metformin *versus* placebo + metformin: liraglutide (subcutaneously once daily) was titrated from 0.6 mg to 1.8 mg within 4 weeks and metformin (orally) was titrated from 500 mg twice daily to 1 g twice daily in 4 weeks. The study period for each patient was approximately 26 weeks (12 + 12 weeks with a ≥2 weeks wash-out period) and consisted of 4 major visits (at weeks 0, 12, 14 and 26); the wash-out was between weeks 12 and 14[25].

2.4. Assay

Density profiling of lipoproteins: lipoproteins were pre-stained with a lipophilic fluorescent probe and then separated by continuous density gradient ultracentrifugation. The density profiles of the separated lipoproteins are then imaged and recorded as fluorescence intensity distributions within the ultracentrifuge tube [27]. Based on density, the lipoprotein density profile is divided into subfractions and the lipoprotein density is inversely related to the lipoprotein particle diameter [28], for a detailed description see Supplementary Materials. Measurements of total-, HDL-, and VLDL-cholesterol and triglyceride (mmol/L) were done by absorption photometry (Cobas 8000, Roche), with a CV of 5% and LDL-c calculated a.m. Friedwald. Additionally, we performed *post-hoc* measurements of plasma apoB, to ensure significant correlations to known apoB containing subfractions. ApoB was measured by quantitative ELISA (ELH-ApoB, lot # 110818 2363, RayBiotech Life, Norcross, GA), for a detailed description see Supplementary Materials. However, values were uniformly lower (1/10th) than expected despite apoB is generally considered stable for immuno-assays for two years [29,30]. Nevertheless, apoB values diminished by storage is highly correlated to values in fresh plasma [30], thus correlations between lipoprotein AUC/LDL-c and apoB are valid but not apoB concentrations *per se*.

Plasma hsCRP (mg/L) and insulin (pmol/L) were determined by enzyme-linked immunosorbent assay (ELISA) (Siemens Healthcare Diagnostics, LA, California, USA); for hsCRP: intra-assay CV of 2.8–8.7% and inter-assay CV of 3.1–8.7%, and for insulin: intra-assay CV of 3.3–5.5% and inter-assay CV of 4.1–7.3%. TNF-α (pg/mL) was determined by ELISA (DRG Instruments, Marburg, Germany): an intra-assay CV of 3.3–4.5% and an inter-assay CV of 6.3–6.6%. NEFA (mmol/L) was determined by enzymatic test (Wako Chemicals, Neuss, Germany): intra-assay variance of 1.5% (median) and inter-assay 7.5% (median).

2.5. Statistical analysis

Data are reported as median (interquartile range, IQR, i.e. 25th; 75th percentile) or mean ± SD. Student paired *t*-test was used for normally distributed data in groups comparisons. In non-normally distributed data, Wilcoxons Sign Rank test was used. Lipid fraction levels were skewed, therefore, in Table 2, the *"Difference"* column is not the result of subtractions of the median values in the preceding columns named *"Placebo + Metformin"* and *"Liraglutide + Metformin",* but is the median of subfractions on subject level values. A two-sided value $p < 0.05$ was considered statistically significant. The primary objective of this paper was intention to treat (ITT) analysis of the effect on lipoprotein density and markers of low-grade inflammation. ITT-analysis was performed utilizing all available data from patients from the intention to treat population, including patients with missing data due to drop-out during the trial (Fig. 1), drop-outs contributed with whatever available data. Data from the ITT-population were furthermore tested for the possibility of a carry-over effect between treatment periods. Additionally, per-protocol analysis was done to explore subgroup effects following division into "liraglutide-first" and "placebo-first" groups. Sample size calculation was published earlier and was based on the primary endpoint of the study (Beta-cell function expressed as disposition index) [25,31], however, *post-hoc* power analysis was performed based on the actual data in the present paper. With n = 28 patients in paired analysis and $p < 0.05$, the SD for the difference between two values from the placebo period was used: total-lipid SD = 2378, $total_{LDL}$ SD = 1568 and LDL_5 SD = 795 and provided a power of 80% to detect a minimum detectable difference of total-lipid: 1307.8, $total_{LDL}$: 862.3 and LDL_5: 437.2. The same analysis was done for total-cholesterol, HDL-c, LDL-c, VLDL-c and triglyceride; SD for the differences in the placebo period used were: 0.91, 0.13, 0.62, 0.19 and 2.38 and provided a power of 80% to detect a minimum detectable

Table 1
Baseline characteristics of the ITT-population.

Variable	Baseline
Age (years)	62.3 ± 7.6
Males n (%)	31 (79)
Waist (cm)	110.4 ± 11.2
Waist/hip ratio	1.0 ± 0.1
Weight (kg)	96.9 ± 17.1
BMI (kg/m^2)	31.6 ± 4.8
Fasting glucose (mmol/L)	6.9 (6.1; 7.4)
Fasting glucose (mg/dl)	123 (109; 133)
HbA1c (%)	6.4 ± 0.5
HbA1c (mmol/mol)	47 ± 6
HOMA-IR	4.9 (3.0; 7.5)
TNF-α (pg/mL)	6.1 (5.3; 7.0)
CRP (mg/L)	2.72 (1.15; 3.85)
Cholesterol (total) (mmol/L)	4.5 ± 1.3
HDL-cholesterol (mmol/L)	0.99 ± 0.24
LDL-cholesterol[a] (mmol/L)	2.4 ± 0.8
VLDL-cholesterol (mmol/L)	0.9 ± 0.4
Triglyceride (mmol/L)	1.65 (1.27; 2.42)
Statins (n (%))	37 (95)
Pre-study ADT[b] (n (%))	
Metformin	15 (38)
Sulfonylurea	1 (3)
Lifestyle intervention only	24 (62)

Data are expressed as median (IQR, 25th; 75th percentiles) or mean ± SD. Baseline values are based on n = 39.
BMI; body mass index, HOMA-IR; homeostasis model-insulin resistance, TNF-α; tumor necrosis factor-α, CRP; C-reactive protein.
[a] a.m. Friedwald.
[b] Anti diabetic treatment.

difference of total-cholesterol (0.5 mmol/L), HDL-c (0.07 mmol/L), LDL-c (0.34 mmol/L), VLDL-c (0.1 mmol/L) and triglyceride (1.31 mmol/L).

Statistical analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Written informed consent was obtained from each patient included in the study and the study was approved by the Regional Committee on Biomedical Research Ethics of the Capital Region of Denmark and was carried out in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP) standards. The study protocol conforms to the guidelines of the Declaration of Helsinki. The protocol was registered at Clinicaltrials.gov with ID: NCT01595789 [25].

3. Results

3.1. Participants

Of the 41 patients randomized, two declined to participate before the first visit and nine discontinued the study; twenty-eight patients completed all study visits (Fig. 1). Baseline characteristics of the 39 patients are presented in Table 1. At baseline, 95% of the patients were on statin therapy and only in one patient dosage was changed during the study. Data from this patient is excluded.

3.2. Lipoprotein density

Total lipoprotein mass (AUC) was significantly reduced by metformin/placebo: −1382.9 (−3287.5; 816.8), $p = 0.003$, and by metformin/liraglutide: −816.9 (−2775.9; 363.3), $p = 0.02$, however, with no difference between treatments ($p = 0.9$), Table 2. Total LDL was reduced by metformin/placebo and metformin/liraglutide treatments; −515.1 (−1707.3; 775), $p = 0.04$ *vs.* −528.4 (−1707; 137.3), $p = 0.03$, with no significant difference between treatments ($p = 0.8$). The reduction of total LDL AUC during metformin/liraglutide treatment was driven by a significant reduction in LDL_5 of −399.6 (−805.9;

Table 2
Change in LDL and HDL and subfraction AUCs relative to baseline values in the ITT-population.

	Density (mg/dL)	Particle diameter (Å)	Baseline	Placebo + metformin	*p*	Liraglutide + metformin	*p*	Difference	*p*
TRL	< 1019		1586.5 (1167.3; 2821.1)	−111.9 (−417.1; 109.8)	0.1	−48.8 (−621.5; 99)	0.3	39.3 (−230.5; 434.5)	0.7
LDL_1	1019–1023	270	354 (310.4; 432.5)	−16.3 (−65.1; 31.9)	0.2	−32.4 (−62.2; 14.3)	0.4	−4.9 (−106; 112.1)	0.7
LDL_2	1023–1029	255	575.8 (486.8; 755.6)	−43.5 (−112.3; 75.8)	0.2	−47.5 (−135; 23.4)	0.06	−34.9 (−155.7; 131)	0.6
LDL_3	1029–1039	247	1175.8 (1019.3; 1512.3)	−65.2 (−176.2; 90.5)	0.2	−169 (−247.5; 78.2)	0.06	−134.2 (−307.2; 227.6)	0.5
LDL_4	1039–1050	237	2530.1 (1799.2; 3244)	−63.5 (−536.4; 366.3)	0.4	−67.5 (−542.5; 452)	0.8	−21.3 (−336.8; 468.9)	0.9
LDL_5	1050–1063	226	2315.2 (1896.3; 3367.9)	−221 (−668.8; 311.9)	0.1	−399.6 (−805.9; 44.9)	0.01	−295.9 (−846.8; 580.7)	0.4
Total LDL			6922 (6070.4; 8869.2)	−515.1 (−1707.3; 775)	0.04	−528.4 (−1707; 137.3)	0.03	−66 (−1562.9; 826.6)	0.8
HDL_{2b}	1063–1091	111	2328.9 (1902.7; 2925.8)	−29.9 (−290.8; 106.5)	0.2	−173.2 (−380.2; 164.3)	0.2	−172.8 (−392.2; 423.2)	0.8
HDL_{2a}	1091–1110	93	2624 (2213.8; 3130.9)	−108.5 (−529.5; 131.5)	0.04	−236.5 (−482.5; 94.7)	0.07	−153.9 (−558.4; 387.8)	0.7
HDL_{3a}	1110–1133	85	2943.5 (2620.6; 3227)	−48 (−505.3; 179.3)	0.3	−145 (−333.5; 60.4)	0.1	−106.5 (−421.7; 190.5)	0.4
HDL_{3b}	1133–1156	80	1253.1 (1093.7; 1528.3)	−40.1 (−163.6; 78)	0.1	−27.2 (−206; 168.6)	0.5	−0.8 (−238.4; 272.2)	1
HDL_{3c}	1156–1179	75	758.7 (659.3; 863.5)	52.2 (−219.2; 185)	1	−5.6 (−179.1; 74)	0.4	−29 (−265.3; 237)	0.7
Total HDL			10158 (8843.2; 11149)	−498.3 (−1002.4; 275.4)	0.01	−561.7 (−1261.2; 663.2)	0.07	−168 (−1247.5; 669)	0.5
Total			19079.5 (17137.9; 21466.3)	−1382.9 (−3287.5; 816.8)	0.003	−861.9 (−2775.9; 363.3)	0.02	−273 (−2487.2; 2845.3)	0.9

Values presented as medians (25th; 75th percentiles).
TRL; triglyceride rich lipoprotein. Å; Ångström.

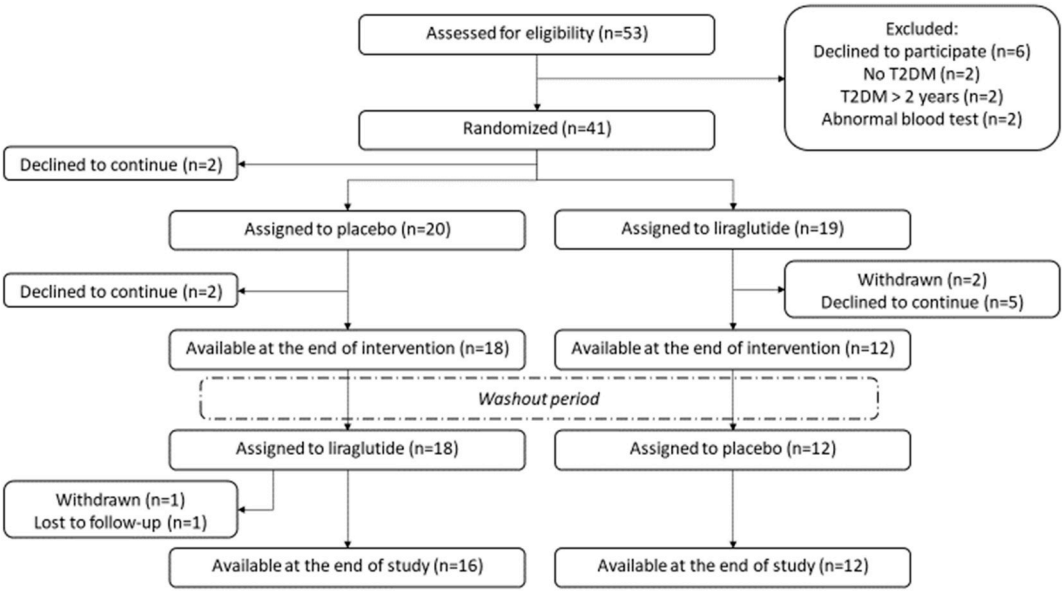

Fig. 1. Flow chart of the course of the study.

44.9), $p = 0.01$, whereas LDL_5 lowering in metformin/placebo was about half this amount and not significant, $p = 0.1$, nevertheless, the difference between treatment arms was not significant ($p = 0.4$) (Table 2). Levels of triglyceride-rich lipoproteins (TRL) were not affected by either treatment. Metformin/placebo also reduced total HDL AUC significantly, −498.3 (−1002.4; 275.4), $p = 0.01$, primarily by reduction of HDL_{2a}. Addition of liraglutide had no additional effects on HDL levels or produced a significant difference between treatments (Table 2).

At baseline, all patients had the atherogenic LDL pattern B, which is characterized by a preponderance of LDL particles ≤255 Å (small dense particles) [32]. The amount of lipoprotein in *pattern B* was reduced following metformin/placebo treatment by −485.3 (−1656.5; 714.5) $p < 0.05$ and by metformin/liraglutide by −492.2 (−1774.6; 29.5) $p = 0.02$, but with no difference between groups (−18.1 (−1456.9; 849.9), $p = 0.8$).

Additionally, HDL2/HDL3 ratio was calculated; baseline ratio 1.05 (0.8; 1.19) was not affected by either treatment, hence difference between treatment was insignificant; −0.03 (−019; 0.21), $p = 0.9$.

3.3. Cholesterol and triglyceride levels

Baseline levels (Table 1) were not changed by liraglutide treatment apart from HDL-c, which was reduced by −0.08 (0.19) mmol/L ($p = 0.03$). However, the combination of metformin and liraglutide caused minor but significant reductions in total-c, HDL-c, LDL-c, VLDL-c and triglyceride levels. Of notice, reductions in total-c was −0.6 (1.0) mmol/L, $p = 0.001$ and LDL-c was −0.3 (0.7) mmol/L, $p = 0.02$, but with no difference between groups (Supplementary Table A1).

3.4. Subgroup analysis of liraglutide-first vs. placebo-first

For illustrative purposes, to reveal whether an effect of liraglutide treatment was sustained or would subside during wash-out period, a purely explorative per-protocol subgroup analysis was made. Subgroup analysis of the "liraglutide-first" group revealed that metformin/liraglutide significantly suppressed total-c and LDL-c levels, with a rebound effect during the wash-out phase and non-significant changes during the placebo treatment period (Supplementary Fig. A1). Subgroup analysis comparing the "liraglutide-first" to "placebo-first" groups with regards to lipid subfractions revealed that metformin/liraglutide significantly suppressed the LDL_5 fraction by 16% ($p = 0.03$) during treatment, but the subfraction rebounded significantly ($p = 0.03$) to pre-treatment value at the end of the wash-out period (Supplementary Fig. A2). For comparison, "placebo-first" indicates similar dynamics for LDL subfractions during metformin/placebo treatment, however, changes are insignificant.

3.5. Markers of low-grade inflammation

Baseline TNF-α 6.1 (5.3; 7.0) pg/mL was reduced by metformin/placebo −0.2 (−0.6; 0.2) pg/mL ($p < 0.05$) but was not affected by metformin/liraglutide treatment and with no significant difference between treatments ($p = 0.3$). Baseline CRP 2.72 (1.15; 3.85) mg/L was reduced by metformin/liraglutide treatment −0.5 (−1.58; 0.03) mg/L ($p = 0.01$) but not by metformin/placebo ($p = 0.9$), and with no significant difference between treatments ($p = 0.2$), (Supplementary Table A2).

3.6. Explanatory variables

We observed no correlation between change in TNF-α and change in body weight (R [2] = 0.06, $p = 0.2$) or HOMA-IR ($R^2 = 0.001$, $p = 0.9$) neither did we observe correlations between change in CRP and change in body weight (R [2] = 0.02, $p = 0.5$) or HOMA-IR ($R^2 = 0.05$, $p = 0.2$). We did not find any correlation between reduction of LDL_5 and weight loss ($R^2 = 0.06$, $p = 0.2$) with metformin/liraglutide therapy. However, despite a significant body weight loss (−2.7 kg; $p = 0.004$)[31] by liraglutide treatment, it did not result in a carry-over effect between treatment periods [31], neither with respect to weight loss ($p = 0.45$)[31] nor insulin sensitivity ($p = 0.21$)[31]. Furthermore, we did not find any carry-over effect with respect to LDL subfractions, however, our data indicates that there was a possible carry-over effect in certain HDL subfractions and HDL_{Total} (Supplementary Table A4). Additionally, we did not find any correlations between changes in markers of low-grade inflammation or lipid subfractions.

By multiple regression analysis, the variance in TNF-α could be not be explained by changes in body weight, glucose, NEFA, C-peptide or HOMA-IR (R [2] = 0.14, $p = 0.6$), however, 40%of the variance in CRP was explained by changes in weight, C-peptide, NEFA, glucose and HOMA-IR (R [2] = 0.39, $p = 0.03$). We observed no correlation between changes in CRP and TNF-α ($R^2 = 0.03$, $p = 0.3$).

3.7. Compliance and safety

Compliance to study medication was > 90% of prescribed dosages with no significant differences between treatment periods. Adverse event frequency was higher in the active treatment periods, predominantly due to gastrointestinal side effects [31]. Serious adverse events were observed in a total of 9 cases: 3 in the active period, 4 in the placebo period, and 2 in the wash-out period. A detailed description of adverse events is published elsewhere [33].

4. Discussion

This study aimed to investigate lipid particle sub-fractions in patients at high risk of cardiovascular events, i.e. coronary artery disease combined with type 2 diabetes. Overall, in ITT-analysis, liraglutide did not improve LDL or HDL subfractions as measured by lipoprotein density profiling or LDL-c as measured by absorption photometry. However, the combination of liraglutide and metformin significantly reduced the atherogenic small, dense LDL_5 particles despite the population being well-controlled on stable statin therapy. This apparent association led us to do a per-protocol subgroup analysis, which indicated a significant rebound of LDL_5 particle during washout in the liraglutide first sub-group. These observations could indicate a positive effect on atherogenic processes by this treatment modality, which is supported by a similar rebound effect with respect to LDL-c (Supplementary Fig. A1). However, ITT-analysis did not confirm a significant effect of liraglutide on LDL-subfractions, this is why these apparent associations should be interpreted with caution. We are aware of the risk of introducing bias to this kind of analysis, which introduces a certain risk of false positive outcomes. Analysis on lipoprotein density was not a primary outcome measure in the AddHope2-trial forming the basis for these data [25], however, we decided to investigate the possible effects of liraglutide on lipoprotein densities as an explanation behind the positive cardiovascular outcomes with liraglutide treatment [13]. We find that our data warrants further studies in a larger setting with sufficient power to confirm the indications presented here.

In patients with T2DM and metabolic syndrome, liraglutide reduced total- and LDL-c and triglycerides [34], however, in that population, only 47% were on statin therapy and baseline HbA1c was 8.8% [34]. On the other hand, only the combination of metformin and liraglutide reduced levels of cholesterols and triglyceride in the study present (Supplementary Table A1). A body weight loss can reverse the atherogenic pattern B lipid profile [32], however, liraglutide may only partially improve the atherogenic lipid profile [35] and changes in LDL subfractions with metformin are similar to those seen with intensive lifestyle intervention [36], which is confirmed by comparing our data to the study by Pedersen et al. in a pre-diabetic population similar to ours with CAD [27]. In the *Liraglutide Effect and Action in Diabetes* (LEAD-) studies, LDL-C and triglyceride levels were reduced [37,38], a finding confirmed by an observational study by Russo et al., [15], however, none of these studies examined density profiles.

Additionally, data from observational real-world clinical studies demonstrate that the effects of liraglutide treatment are consistent with clinical trial findings [43,44], in which liraglutide was used as add-on treatment and showed superior effects in respect to comparators [45] also with regards to total-c, LDL-c and triglyceride [46]. However, in general, patients in these real-world studies are more obese and not as well-treated with statins as compared to our study [43,46], thus we suggest that the real world effect of liraglutide on lipid subfractions could be even more pronounced.

A predominance of small, dense LDL has been accepted as a cardiovascular risk factor [39] and an interesting finding in our study is the significant reduction in the most atherogenic subunit, LDL_5, following liraglutide in combination with metformin, although not significant compared with metformin and placebo. Additionally, we find strong correlations between LDL_{Total} and pattern B to LDL-c, and significant correlations between ApoB and LDL_{Total} and its subfractions. Of interest is the observed rebound effect of LDL_5 in the wash-out period of the present study, which indicates that liraglutide in combination with metformin therapy may be capable of improving the most atherogenic subfraction, even though the patient is on stable statin therapy, an effect which was not correlated to the weight loss. Despite the cross-over design, the presence of a carry-over effect was ruled out in the present study, both with respect to weight loss or insulin sensitivity [31] and with regards to LDL subfractions.

Among patients with previous myocardial infarction, TNF-α levels are persistently elevated, suggesting an inflammatory instability present in stable patients at increased vascular risk, and therapies that attenuate this inflammation may represent a new direction in the treatment of CAD [20]. Previous findings in cardiovascular high risk patients with T2DM showed a reduction on TNF-α levels by liraglutide [23], contrasting our data in which no effect is found. Although levels of CRP are closely associated with obesity and insulin resistance [40], effects of incretin based therapy are ambiguous and the beneficial effects seem independent of changes in body weight [41]. Nevertheless, in patients with pre-diabetes or early T2DM liraglutide, it is superior to lifestyle intervention in reducing CRP levels, despite a comparable reduction in BMI [42], which is in accordance with our results.

4.1. Limitations

The present explorative per protocol analysis may induce a risk of both type I and type II errors, because the endpoints tested were not predefined outcomes in the AddHope2-trial [25], and secondly, we risk this setup to be underpowered. Nevertheless, ITT-analysis indicates an additional effect of liraglutide/metformin added to stable statin treatment in this population of patients with T2DM and CAD. Furthermore, per-protocol analysis implies an immediate effect on lipoprotein levels in patients undergoing 12-week treatment with liraglutide. During the trial, we had a drop-out rate of 13 patients, which could cause a negative impact on the results, however, n = 2 withdraw consent after randomization but before the first visit, and n = 4 withdraw their consent shortly after visit one due to the extensive trial examination course [25], not due to study medication adverse events. Despite these conditions, it is consistent with clinical trials that the drop-out rate is prominent in the liraglutide group [13,47,48].

In conclusion, liraglutide and metformin compared to placebo and metformin did not significantly improve LDL subfractions or LDL-c, but the combination of liraglutide and metformin indicated an improvement in the atherogenic LDL Pattern B, notably by reducing the small dense subfraction, LDL_5, in patients with CAD and newly diagnosed well-controlled T2DM on stable statin therapy. This combination therapy also reduced CRP levels but with no effect on TNF-α.

Conflicts of interest

CA: advisory board and lecture fees: Novo Nordisk. LRP: share owner: Novo Nordisk. OWN: funding of educational and research tasks from ResMed and participated on advisory boards for Novartis. SM: advisory boards: AstraZeneca; Boehringer Ingelheim; Bristol-Meyers Squibb; Eli Lilly; Intarcia Therapeutics; Johnson & Johnson; Merck Sharp & Dohme; Novartis; Novo Nordisk; Sanofi Aventis. Lecture fees: AstraZeneca; Boehringer Ingelheim; Bristol-Meyers Squibb; Eli Lilly; Merck Sharp & Dohme; Novartis; Novo Nordisk; Sanofi Aventis. Research Grant Recipient: Novo Nordisk. A.Sajadieh: advisory board and lecture fees: Novo Nordisk. SBH: has received funding of educational and research tasks from Novo Nordisk, Abbott, Eli Lilly, Pfizer, Boehringer Ingelheim, Bristol-Meyers Squibb, and Merck Sharp & Dohme. Th other authors have nothing to disclose.

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Author contributions

CA: acquired data, performed the statistical analyses, interpreted data, and drafted and revised the manuscript for important intellectual content and approved the final version. PK, LRP: acquired data, interpreted data, and revised the manuscript for important intellectual content and approved the final version. A.Samkani: interpreted data, and revised the manuscript for important intellectual content and approved the final version. RLW, MF: performed biochemical analysis, interpreted data, and revised the manuscript for important intellectual content and approved the final version. OWN, OPK, SM: interpreted data and revised the manuscript for important intellectual content and approved the final version. SBH, A.Sajadieh: conceived and designed the study, interpreted data, and revised the manuscript for important intellectual content and approved the final version.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.07.007.

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