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ORIGINAL ARTICLE

Treatment with liraglutide may improve markers of CVD reflected by reduced levels of apoB

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Summary

Background

Dislipidaemia and increased levels of apolipoprotein B (apoB) in individuals with obesity are risk factors for development of cardiovascular disease (CVD). The aim of this study was to investigate the effect of weight loss and weight maintenance with and without liraglutide treatment on plasma lipid profiles and apoB.

Methods

Fifty-eight individuals with obesity (body mass index 34.5 ± 3.0 kg/m^2 [mean ± SD]) were included in this study. After 8 weeks on a very low-calorie diet (800 kcal/day), participants were randomized to weight maintenance with meal replacements with or without liraglutide (1.2 mg daily) for 1 year. Plasma samples from before and after weight loss and after 1 year of weight maintenance were subjected to nuclear magnetic resonance-based lipidomics analysis.

Results

After an 8-week low-calorie diet, study participants lost 12.0 ± 2.9 kg (mean ± SD) of their body weight, which was reflected in their lipid profiles (80 out of 124 lipids changed significantly), including reduced levels of apoB, total cholesterol, free cholesterol, remnant cholesterol, triglycerides, low-density lipoprotein and very low-density lipoprotein subclasses. After 1 year of maintained weight loss, the majority of the lipids had returned to pre-weight loss levels even though weight loss was successfully maintained in both groups. Interestingly, apoB levels remained low in the liraglutide treated group (apoB change: 0.03 ± 0.02 mmol/L, $p = 0.4$) in contrast to an increase in the control group (apoB change: 0.06 ± 0.07 mmol/L, $p = 0.02$).

Conclusion

An 8-week low-calorie diet, in individuals with obesity, reduced plasma levels of lipids and the atherogenic marker apoB. After 1 year of weight maintenance, only study participants treated with liraglutide maintained reduced levels of apoB, despite similar body weight maintenance. Treatment with liraglutide may therefore reduce apoB levels and thus reflect lower CVD risk. Including apoB measurements in clinical practice when monitoring patients with dislipidemia or CVD might prove to be useful.

Keywords: apoB, liraglutide, metabolomics, weight loss.

Introduction

The prevalence of obesity is increasing and contributes significantly to the pathogenesis of cardiovascular disease (CVD) (1–3). Individuals with obesity often have an altered composition of circulating plasma lipids (termed dislipidemia), which includes increased levels of apolipoprotein B (apoB), decreased levels of high-density lipoprotein (HDL) and altered particle composition of low-density lipoprotein (LDL) (4,5). Increased plasma levels of apoB seem to independently serve as a risk marker for CVD (4,6–8).

Levels of apoB have thus been suggested to be a superior predictor of CVD compared with levels of LDL because of the 1:1 ratio of apoB molecules and number of atherogenic lipoprotein particles (9–12). Measurement of apoB levels therefore provides information about all the atherogenic lipoproteins (very-LDL [VLDL], LDL and IDL) whereas measurement of only LDL levels does not account for VLDL and IDL levels. Individuals with obesity, metabolic syndrome and/or type 2 diabetes (T2D) frequently have increased levels of apoB despite relatively more normal LDL concentrations (13,14). Furthermore, these patient groups also have a two-fold to fourfold increased risk of CVD (15–17). An increased concentration of apoB and thus an increased amount of apoB-containing lipoproteins (VLDL, IDL and LDL) may trigger the process of atherosclerosis (10). Additionally, elevated levels of apoB have been linked with structural vascular changes including enlarged carotid intima-media thickness and increased arterial stiffness (18), all of which are risk factors of cardiovascular morbidity and mortality (18).

The glucagon-like peptide-1 receptor agonist (GLP-1RA) liraglutide, used in the treatment of T2D (19,20), has been approved for weight loss management in individuals with obesity (21) as it, together with low-calorie diet, induces a metabolically relevant sustained weight loss (~8–10 kg) by inhibiting appetite (22,23). Furthermore, treatment with liraglutide decreases cardiovascular mortality in individuals with T2D (the recent published LEADER study (26)); however, the underlying mechanism remains unknown.

Interestingly, GLP-1 has been suggested to act as a regulator of intestinal lipid absorption through downregulation of apoB48 (24–26), but neither the effect of long-term GLP-1RA treatment on lipid metabolism nor its effect on the plasma levels of apoB in weight-reduced non-diabetic individuals with obesity have been evaluated.

Thus, we hypothesized that liraglutide may improve the atherogenic risk profile by reducing plasma concentrations of apoB. To test this, we used a nuclear magnetic resonance (NMR) spectroscopy-based metabolomics to delineate the potential effects of liraglutide on lipid fractions in humans using plasma samples from a previous randomized controlled clinical trial of 58 non-diabetic individuals with obesity (27). This investigation may thus aid to the understanding of one of the mechanisms behind the reduced risk of CVD with GLP-1RA treatment observed with liraglutide (28).

Method

Study participants

Fifty-eight glucose-tolerant individuals with obesity were enrolled in this previously described randomized controlled trial (27). Participants were aged between 18 and 65 years (45.9 ± 1.5 years) and had a mean body mass index of 34.4 kg/m^2 (range 30.0–39.9 kg/m^2). Individuals were excluded from entering the study if they suffered from any acute or chronic diseases. Fifty-two participants completed the 8-week weight loss intervention period, and 42 individuals completed the 1-year study period (22 participants in the liraglutide group and 20 participants in the control group).

Weight loss programme

All participants were initially enrolled in a weight loss intervention programme for 8 weeks with the objective to lose of at least 7.5% body weight. Intake of calories was based on a powdered formula mixture, which provided 810 kilocalories (3,402 kJ) per day. All products were provided by the Cambridge Diet (Cambridge Weight Plan). Standard recommendations for intake of essential amino acids, fatty acids, vitamins and minerals were met with this diet. Daily intake of protein was at least 43.2 g, and intake of essential fatty acids and linoleic acid was 3.0 and 0.4 g, respectively.

Randomization and weight maintenance programme

After 8 weeks on the low-calorie diet, the participants were randomized into two groups: one receiving a daily subcutaneous injection of liraglutide 1.2 mg daily (Flexpen device, Victoza, Novo Nordisk A/S, Bagsværd, Denmark) and another group serving as a control group. Both groups followed the Cambridge Weight Loss Maintenance Program along with calorie restriction (calorie need subtracted by 600 kcal) for the entire study period of one year. Furthermore, study participants received individual guidance on diet and exercise habits from a dietician following official Danish guidelines. If the participants experienced weight gain during the weight maintenance period, they were allowed to replace up to two meals per day with Cambridge Weight Plan products to ensure a stable weight. Both groups maintained the weight loss achieved in the initial weight loss programme (weight change in liraglutide group 0.49 ± 6.84 kg [mean ± SD] and in control group 2.23 ± 7.32 kg), and there was no significant weight difference between the groups after 1 year. However, the control group replaced on an average one meal per day with a low-calorie diet meal compared with no replacements in the liraglutide group (liraglutide group compared with control group, minus one meal per day (95% CI=−0.6 to −1), $P<0.001$) as previously described (27).

Blood samples after an overnight fasting were obtained before weight loss (screening), at baseline (after weight loss/randomization) and 1 year after baseline.

Metabolite quantification

Plasma samples were analysed using a high-throughput NMR-based platform (29). Lipids and their related metabolites (hereof lipid particles) in blood samples obtained at screening (before weight loss), baseline (after weight loss) and after 1 year of weight maintenance were detected. The high-throughput NMR platform (Nightingale Health Ltd, Helsinki, Finland) has previously been used in various epidemiological and genetic studies, and details of the experimental protocols, including sample preparation and spectroscopy, are described in references (29–33).

Statistical analyses

Statistical analyses were performed using R, version 3.1.3 (http://www.r-project.org).

All metabolites were log base 10-transformed prior to analysis to obtain normal distribution. Changes in metabolite concentrations within each group were analysed with Students Paired *t* tests. Changes in metabolite concentrations at each time point were compared between liraglutide and control group with a general linear model adjusted for sex, age and delta values for weight change or with an unpaired *t*-test. Data are shown as mean ± SD. To account for multiple testing, a false discovery rate (FDR) of 5% was used. *P* values < 0.026 were considered significant.

Ethics

The study was approved by the Ethical Committee in Copenhagen (reference number: H-4-2010-134) and was performed in accordance with the Helsinki Declaration II. Participation in the trial was voluntary, and the participants could retract their consent to participate at any time. ClinicalTrials.gov Identifier: NCT02094183.

Results

Clinical characteristic of the study population

The two groups were comparable in terms of gender distribution (women/men ratio: liraglutide group: 85%/15%; control group: 92%/8%; $p = 0.7$), body weight (liraglutide group: 98.6 ± 13.5 kg; controls: 96.8 ± 9.6 kg, $p = 0.6$) and body fat percentage (liraglutide group: 41.4 ± 5.8; controls: 42.4 ± 4.7, $p = 0.5$) (Table 1).

Lipid profile during weight loss intervention

The 12 kg weight loss was associated with a global shift in the composition of plasma lipids with significant changes in 80 out of the 124 measured lipid fractions (Table **S1**). Levels of both apoB (0.98 to 0.84 mmol/L, $p = 1.8 \times 10^{-4}$) and apoA1 (1.57 to 1.44 mmol/L, $p = 1.1 \times 10^{-5}$) decreased significantly. Furthermore, plasma concentration of total cholesterol (2.27 to 2.05 mmol/L, $p = 1.5 \times 10^{-4}$), ester cholesterol (3.18 to 2.76 mmol/L, $p = 0.0002$), free cholesterol (1.34 to 1.24 mmol/L, $p = 0.02$), remnant cholesterol (1.61 to 1.36 mmol/L, $p = 0.0003$) and plasma triglycerides (1.46

Table 1 Demographic characteristics of study population

Characteristic	Screening	Baseline (randomization)			One year		
	Inclusion *N* = 58	Liraglutide group *N* = 22	Control group *N* = 20	*p*-value	Liraglutide group	Control group	*p*-value
Women	47 (81%)	17 (77%)	19 (95%)	0.19	17 (77%)	19 (95%)	0.19
Men	11 (19%)	5 (23%)	1 (5%)	0.19	5 (23%)	1 (5%)	0.19
Body weight	97.6 ± 11.8	88.1 ± 11.7	82.8 ± 7.5	0.09	87.85 ± 13.38	85.02 ± 10.4	0.45
BMI (kg m^{-2})	34.4 ± 2.8	31.3 ± 3.2	29.1 ± 2.0	0.01	31.15 ± 4.16	29.81 ± 3.11	0.25
Waist (cm)	109.2 ± 10.2	100 ± 9.7	95.3 ± 7.3	0.09	100.15 ± 14.28	96.35 ± 10.21	0.33
Waist:Hip ratio	0.92 ± 0.08	0.89 ± 0.08	0.86 ± 0.06	0.18	0.89 ± 0.10	0.85 ± 0.07	0.14
Total body fat %	41.9 ± 5.2	39.1 ± 6.3	39.5 ± 4.8	0.82	37.98 ± 6.91	38.5 ± 5.36	0.79
Delta value weight loss (screening to baseline and to one year)	-	−12.0 ± 3.2	−12.1 ± 2.5	0.91	2.23 ± 6.97	0.50 ± 6.69	0.42

Data are expressed as *n* (%) or mean ± SD.
BMI, body mass index.

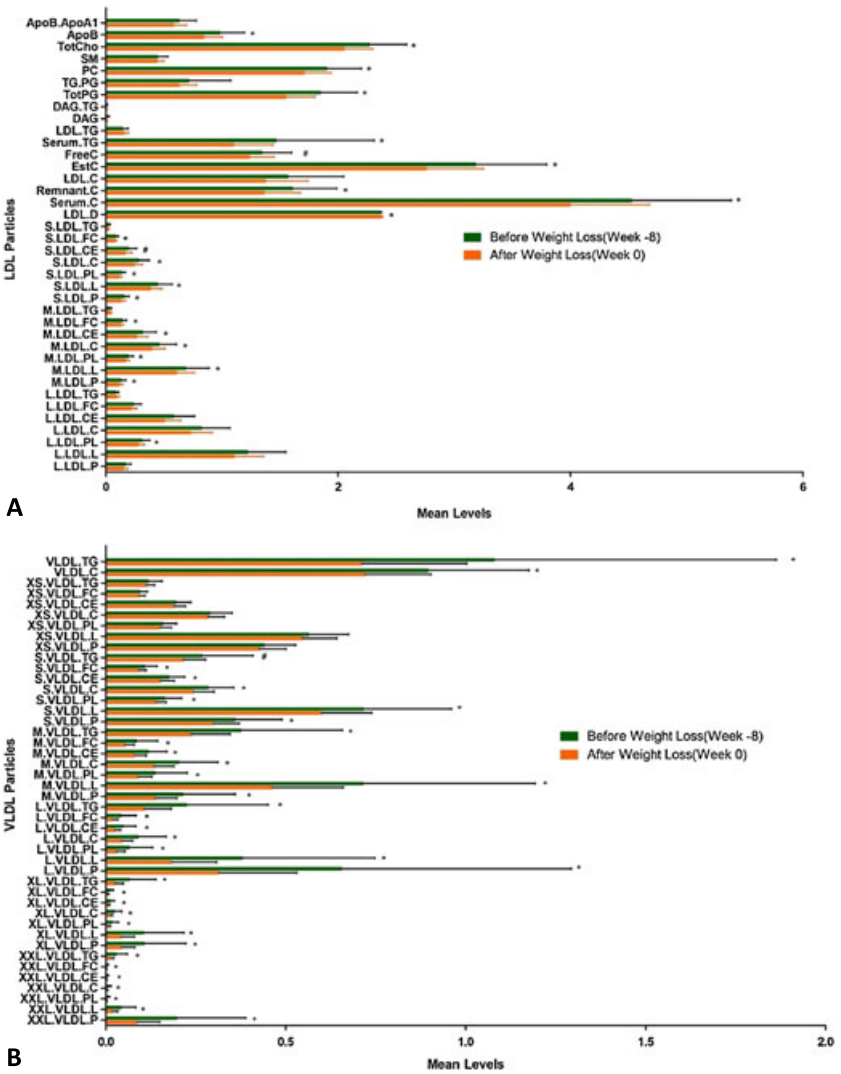

Figure 1 The effect of weight loss on low-density lipoprotein (LDL), very-LDL [VLDL] and high-density lipoprotein (HDL) particles. Bars represent the change in mean lipid concentration (error bar in SD) before weight loss (week −8) and after weight loss (week 0). (A) represents plasma lipids, LDL and subclass particle; (B) represents VLDL subclass particle; and (C) represents HDL and subclass particle. XXL_VLDL_P is expressed in nmol, XL_VLDL_P is expressed in μmol and multiplied by 100, L_VLDL_P is expressed in μmol and multiplied by 100, M_VLDL_P is expressed in μmol and multiplied by 10, S_VLDL_P is expressed in μmol and multiplied by 10, XS_VLDL_P is expressed in μmol and multiplied by 10, L_LDL_P is expressed in μmol, M_LDL_P is expressed in μmol, S_LDL_P is expressed in μmol, XL_HDL_P, L_HDL_P, M_HDL_P and S_HDL_P were multiplied by 100. LDL-D was divided by 10. Except previously mentioned lipids, others are in mmol/L. *$p < 0.026$.

to 1.10 mmol/L, $p = 0.009$) were significantly reduced after the weight loss intervention. Also, significant changes in plasma concentrations of numerous subclasses of LDL, VLDL and HDL particles were observed (Figure 1A and B).

Liraglutide modifies levels of apolipoprotein B during 1 year of weight maintenance

Lipid estimates showed an overall increasing tendency, involving subclasses of HDL, LDL and VLDL particles. The increase in lipid fractions were seen in both groups (Figure 2A and B, Table S2).

All VLDL and LDL particles increased after 1 year of weight maintenance in the control group whereas, and in contrast to, in the liraglutide-treated group, only L-VLDL and the content of triglycerides in S-VLDL increased (Table S2). For HDL-related particles, the control group showed increased plasma levels of HDL3-C, apoA1, S-, M-, L- and XL-HDL-C levels, and in the liraglutide group, plasma levels of HDL3-C, apoA1 and S- and M- HDL-C levels and decreases in HDL-TG content and density were observed (Figure 2, Table 2). In spite of an increase in HDL levels in both groups, the liraglutide group had significantly lower HDL3-C, S-, M- and XL-HDL levels than the control group (Figure 2, Table S2).

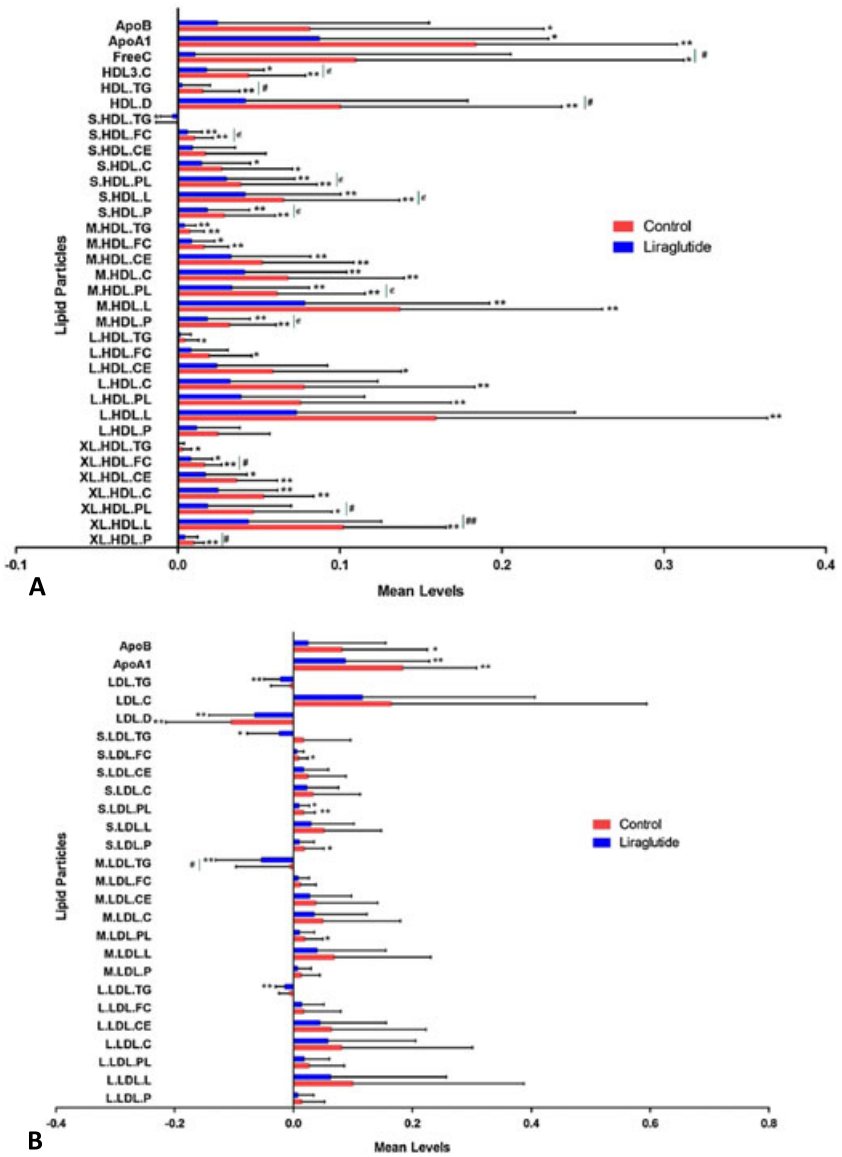

Figure 2 (A and B) The effect of weight maintenance on low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles. Bars represent mean changes (error bar in SD) in lipid concentration (52 to 0 weeks) in LDL particle and subclass. L.LDL.P, M.LDL.P and S.LDL.P expressed in μmol/L multiplied by 1,000; M.LDL.TG expressed in mmol/L multiplied by 10. *p values for the concentration of lipid particle during weight maintenance period at 52 weeks (calculated between 0 and 52 weeks). *p values < 0.026, **p value < 0.01. #Significant difference between liraglutide and control group at 52 weeks follow-up, p values < 0.026.

Furthermore, in the control group, an increase in total plasma cholesterol (+11%, $p = 0.005$), remnant cholesterol (+8%, $p = 0.04$), HDL-C (total cholesterol in HDL) (+17%, $p = 0.0009$), total cholines (+12%, $p = 1.6\text{x}10^{-5}$), ester cholesterol (+14%, $p = 0.002$), free cholesterol (+6%, $p = 0.04$), apoA1 (+12%L, $p = 4.9\text{x}10^{-6}$) and apoB (+7%, $p = 0.025$) was observed despite maintained weight loss. Similar results were observed in the liraglutide group with increases in cholesterol (+9%, $p = 0.04$), HDL-C (+8%, $p = 0.01$), total cholines (+8%, $p = 0.004$), ester cholesterol (+12%, $p = 0.005$) and apoA1 (+6%, $p = 0.01$). However, levels of remnant cholesterol ($p = 0.19$), free cholesterol ($p = 0.99$) and apoB ($p = 0.36$) did not increase significantly in the liraglutide group (Figure 2, Table S2).

Discussion

Low-calorie diet-induced weight loss resulted in a global beneficial change in the plasma lipid profile of individuals with obesity. Furthermore, the cardio-beneficial reductions in apoB, LDL and triglycerides achieved by weight loss

Table 2 Biochemical measures in study population

	Liraglutide				Control group		
Variable	Inclusion	Randomization	One year	*p*-value	Randomization	One year	*p*-value
Serum Cholesterol (mmol/L)	4.53 ± 0.86	4.12 ± 0.58	4.42 ± 0.76	0.037	3.87 ± 0.75	4.40 ± 0.90	0.005
Triglycerides (mmol/L)	1.5 ± 0.84	1.12 ± 0.32	1.09 ± 0.44	0.395	0.99 ± 0.28	1.17 ± 0.84	0.519
VLDL-C (mmol/L)	0.90 ± 0.28	0.74 ± 0.17	0.77 ± 0.20	0.443	0.68 ± 0.17	0.76 ± 0.30	0.083
VLDL-D (nm)	36.96 ± 1.57	35.96 ± 0.85	36.20 ± 1.01	0.197	35.75 ± 0.72	36.19 ± 1.48	0.088
LDL-C (mmol/L)	1.57 ± 0.48	1.45 ± 0.33	1.57 ± 0.41	0.090	1.29 ± 0.38	1.46 ± 0.47	0.138
LDL-D (nm)	23.62 ± 0.12	23.73 ± 0.08	23.67 ± 0.07	0.0005	23.77 ± 0.10	23.67 ± 0.11	0.0004
HDL-C (mmol/L)	1.35 ± 0.28	1.25 ± 0.15	1.36 ± 0.24	0.014	1.28 ± 0.23	1.51 ± 0.32	0.0009
HDL-D (nm)	9.95 ± 0.23	9.99 ± 0.15	10.04 ± 0.21	0.170	9.98 ± 0.15	10.08 ± 0.22	0.0046
HDL2.C (mmol/L)	0.84 ± 0.27	0.77 ± 0.15	0.86 ± 0.22	0.036	0.81 ± 0.19	0.99 ± 0.29	0.073
HDL3.C (mmol/L)	0.51 ± 0.04	0.48 ± 0.03	0.50 ± 0.04	0.031	0.48 ± 0.05	0.52 ± 0.05	3.14×10^{-5}
ApoA1 (g/L)	1.57 ± 0.16	1.44 ± 0.10	1.53 ± 0.15	0.0096	1.44 ± 0.15	1.63 ± 0.19	4.89×10^{-6}
ApoB (g/L)	0.98 ± 0.21	0.87 ± 0.15	0.89 ± 0.17	0.364	0.80 ± 0.15	0.88 ± 0.22	0.025
ApoBApoA1	0.63 ± 0.15	0.61 ± 0.10	0.59 ± 0.12	0.180	0.56 ± 0.10	0.55 ± 0.16	0.370

Data are expressed as mean ± SD. D represents diameter of particles. C represents the content of cholesterol within each lipoprotein particle. *p* values are calculated by paired *t* tests comparing lipid levels at randomization and 1 year follow-up. False discovery rate (FDR) corrected significance p-value < 0.02.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-LDL.

were maintained by liraglutide treatment for 1 year. Thus, treatment with liraglutide maintained low levels of apoB, which may contribute to explain the reduced cardiovascular mortality reported in liraglutide-treated subjects with T2D (28). Changes in apoB levels were related to the parallel changes in the atherogenic lipids: LDL, VLDL and IDL. Despite a maintained weight loss, an overall tendency of increasing lipid particles towards pre-weight loss levels, mainly in the control group, indicating that the effect of a weight loss on lipid particles may only be acute and transient.

We demonstrated that individuals with obesity treated with liraglutide after weight loss were able to sustain a weight loss-induced lowering of apoB levels in contrast to a control group. Interactions between GLP-1RA and levels of apoB have also been reported by others (24,26,34–37), but the mechanism(s) underlying the effect of liraglutide on apoB levels has remained unknown. First, both groups sustained a comparable weight loss (~12 kg) during the 52-week weight maintenance phase, indicating that the apoB-lowering effect of liraglutide is independent of weight loss. Second, liraglutide may also directly affect the dietary uptake of lipids from the intestine or through a reduced hepatic production of lipids. From animal studies, it has been demonstrated that infusion of GLP-1 can reduce the intestinal uptake of lipids (reduced levels of apoB48) and absorption of triglycerides (35). Interestingly, in humans, the GLP-1RA exenatide is able to reduce levels of apoB48 (36,37). Treatment with liraglutide has, similarly to exenatide, been linked with lower levels of total apoB (34) and has been shown to suppress postprandial apoB48 elevation in individuals with T2D (24). This indicates that GLP-1RAs may mediate a reduction in intestinal uptake of fat and thereby influence the risk of atherosclerosis and CVD.

Strengths of this study includes the ability of comparing the effects of liraglutide independent of the effect on weight as both groups successfully maintained the weight loss for 1 year. A limitation of the current study is that the applied NMR-based metabolomics approach cannot differentiate between plasma levels of apoB48 (marker of intestinal fat absorption) and apoB100 (marker of hepatic derived lipoprotein), and we can only conclude that liraglutide affects the total pool of apoB. It would therefore have been of interest to apply a more specific method to analyse changes in the apoB48:apoB100 ratios in subjects treated with a GLP-1RA versus placebo. Future studies, using a more biased approach to assess such changes in plasma levels of apoB48 and apoB100, are therefore needed.

Plasma HDL particles are highly heterogeneous in their physiochemical properties, metabolism and biological activity, and the HDL response is variable and affected both by weight loss and by the type of dietary intervention (38,39). The effect of liraglutide on HDL levels has previously been described by others with diverging directions (40–44). The clinical relevance of lower HDL levels in liraglutide-treated individuals is not completely clarified. HDL-C is a known predictor of CVD; however, the causal link between HDL and atherosclerosis is still uncertain (45). In contrast, we observed lower HDL-C concentrations in liraglutide-treated individuals compared with

controls as well as lower levels of HDL3-C, S- and M-HDL particles. These change may reflect a nutrient-derived attenuation of reverse cholesterol transportation aided by HDL particles, but future studies are needed to clarify this potential mechanism.

Participants in this study were obese, but otherwise healthy and severe dyslipidemia, was not registered in this population. Therefore, it can be speculated that greater changes in lipid profiles, including levels of apoB, may be found in populations with more severe degrees of dislipidemia or known CVD. LDL cholesterol is routinely measured in clinical practice, but as mentioned, LDL measurements have certain disadvantages. The LDL cholesterol concentration does not contain information on other atherogenic lipoproteins, and some patients will develop atherosclerotic disease despite LDL within the normal range (46). Hence, it might be clinically relevant to include measurements of apoB when monitoring patients with dislipidemia and CVD to provide a more accurate lipid profile for risk development of a CVD event.

In conclusion, acute weight loss in individuals with obesity improves the atherogenic lipid profiles, but the changes revert towards screening levels despite weight maintenance. However, with liraglutide treatment, the lowering of apoB levels is maintained, reflecting a change in LDL metabolism, which may contribute to a decreased CVD risk.

Conflicts of Interest Statement

S. M. and J. J. H. have performed consultant services for Novo Nordisk. S. S. T. and T. H. hold stocks in Novo Nordisk. L. E., J. L., N. W. A., Y. M., E. W. I., P. F., A. E. J. and H. V. have no relevant conflict of interest for this study.

Author Contributions

J. J. H., S. S. T. and S. M. planned the study. J. L. and E. W. I. conducted the clinical tests. Y. M and L. E. performed the statistical tests. J. L., L. E., Y. M, N. W. A. and S. S. T intrepreted data. L. E. and J. L. wrote the first draft. All authors participated in editing the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. Metabolite concentrations during acute weight loss intervention.
Table S2. Metabolite concentrations during weight maintenance for 52 weeks.