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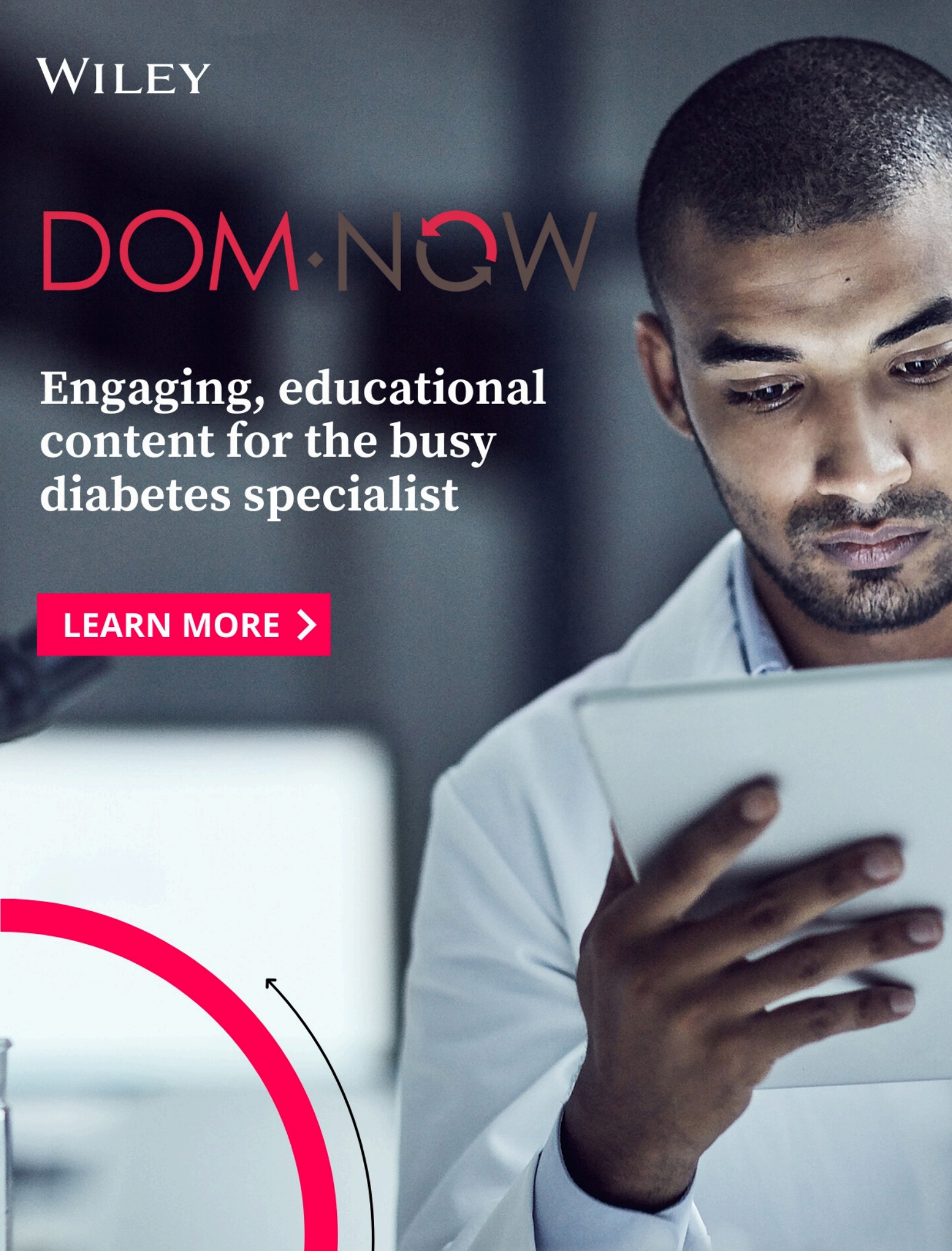

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ORIGINAL ARTICLE

Effect of short-acting exenatide administered three times daily on markers of cardiovascular disease in type 1 diabetes: A randomized double-blind placebo-controlled trial

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Abstract

Aims: To investigate the effect of adding the short-acting glucagon-like peptide 1 receptor agonist (GLP-1RA) exenatide to insulin treatment on markers of cardiovascular risk in type 1 diabetes.

Materials and methods: In a randomized, double-blind, parallel-group trial, 108 individuals with type 1 diabetes aged ≥18 years on multiple daily injection therapy with a body mass index >22.0 kg/m^2 and glycated haemoglobin concentration of 59 to 88 mmol/mol (7.5%–10.0%) were randomized (1:1) to preprandial subcutaneous injection of 10 μg exenatide (Byetta®) or placebo three times daily over 26 weeks as add-on treatment to existing insulin therapy. Reported markers of cardiovascular risk were secondary endpoints and were analyzed in a baseline-adjusted linear mixed model in the intention-to-treat population. The primary results of this study, the MAG1C (Meal-time Administration of exenatide for Glycaemic control in type 1 diabetes Cases) trial, were previously reported.

Results: Exenatide changed total fat mass by −2.6 kg (95% confidence interval [CI] −3.6; −1.6; $P < 0.0001$) and lean body mass by −1.1 kg (95% CI −1.9; −0.4; $P = 0.01$) compared with placebo, as assessed by dual-energy X-ray absorptiometry. Fat mass reductions were similar for central and peripheral fat mass. Exenatide did not change levels of interleukin-2 or -6; tumour necrosis factor-α; C-reactive protein; N-terminal prohormone of brain natriuretic peptide; or 8-oxo-7,8-dihydroguanosine

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(RNA oxidation marker) and 8-oxo-7,8-dihydro-2′-deoxyguanosine (DNA oxidation marker).

Conclusions: Exenatide added to insulin therapy in type 1 diabetes for 26 weeks resulted in body weight loss primarily from fat mass reduction, but had no effect on biomarkers of cardiovascular disease risk.

1 | INTRODUCTION

In type 1 diabetes, cardiovascular disease reduces mean average life expectancy by up to 11 years for men and 13 years for women.[1] Strict glycaemic control through insulin therapy reduces the risk of microvascular and macrovascular complications[2,3] but promotes body weight gain, thus contributing to the growing burden of overweight and obesity in people with type 1 diabetes.[4] Also in type 1 diabetes, weight gain promotes risk of metabolic syndrome and cardiovascular disease.[5–7] Consequently, with fewer than half of patients meeting their recommended glycaemic target,[8] the focus of effective treatment of type 1 diabetes is on both glycaemic control and reduction of cardiovascular disease risk. Glucagon-like peptide-1 (GLP-1) receptor agonist (GLP-1RA) add-on treatment to insulin therapy in type 1 diabetes has been investigated for both long-acting and short-acting compounds and has shown effects with reductions of body weight by 4 to 6 kg and lowering of insulin requirements by up to 9 units (U) per day with some (2–3 mmol/mol) or no improvements in glycaemic control, as assessed by glycated haemoglobin (HbA1c).[9–13] GLP-1RAs exert these effects in type 1 diabetes by glucose-dependently suppressing glucagon release during hyperglycaemia, prolonging gastric emptying rate and reducing food intake.[14] Interestingly, short-acting GLP-1RAs have a sustained decelerating effect on gastric emptying, which translates into sustained improvements in postprandial plasma glucose levels; as opposed to the tachyphylaxis and lack of sustained effect ongastric empying and postprandial glcose levels seen with long-acting GLP-1RAs.[14] To allow assessment of the maximal efficacy of a short-acting GLP-1RA on glycaemic control, administration before each main meal would be of interest.

In type 2 diabetes, some GLP-1RAs have been shown to lower the rate of major adverse cardiovascular events (ie, non-fatal myocardial infarction, non-fatal stroke and cardiovascular-related death) in large cardiovascular outcome trials.[15–17] No GLP-1RA cardiovascular outcome trials exist in type 1 diabetes, and the effect of GLP-1RA treatment on biomarkers of cardiac function and cardiovascular disease, together with biomarkers of oxidative stress in these patients, remains sparsely described. Furthermore, GLP-1RAs' impact on body composition, that is, central and peripheral fat mass loss versus lean mass loss, is not well documented in type 1 diabetes.

In the present study, we evaluated the effect of 26 weeks of treatment with the short-acting GLP-1RA exenatide, administered three times daily (before breakfast, lunch and dinner) as add-on to insulin therapy in patients with type 1 diabetes, on biomarkers of low-grade inflammation and cardiovascular risk (tumour necrosis factor [TNF]-α, interleukin [IL]-2, IL-6, C-reactive protein [CRP] measured as high-sensitivity CRP [hsCRP], and N-terminal prohormone of brain natriuretic peptide [NT-proBNP]), oxidation status (markers of RNA oxidation, 8-oxo-7,-8-dihydroguanosine [8-oxoGuo] and DNA oxidation, 8-oxo-7,8-dihydro-2′-deoxyguanosine [8-oxodG]) and body composition. These endpoints constitute prespecified key secondary endpoints of the MAG1C (Mealtime Administration of exenatide for Glycaemic control in type 1 diabetes Cases) trial (except for markers of oxidative stress).[18] As previously reported, short-acting exenatide did not clinically or statistically significantly reduce the primary endpoint, HbA1c, as compared with placebo after 26 weeks of treatment. Exenatide relevantly lowered prandial insulin requirements by −8.5 U/d (95% confidence interval [CI] −11.2 to −5.7; $P < 0.0001$) or 30% as compared with placebo.[13]

2 | MATERIALS AND METHODS

2.1 | Study design and participants

The MAG1C trial was a 26-week, randomized, double-blind, placebo-controlled, phase 2a clinical trial, testing the efficacy and safety of 10 μg short-acting exenatide administered three times daily as preprandial injections 1 hour of before breakfast, lunch and dinner as add-on treatment to multiple daily injection therapy in type 1 diabetes (www.clinicaltrials.gov, NCT03017352). As previously described,[13] the MAG1C trial was conducted at the Steno Diabetes Center Copenhagen (Gentofte, Denmark); participants were recruited from outpatient clinics in the Capital Region of Denmark. Eligible participants were aged ≥ 18 years with type 1 diabetes (according to World Health Organization criteria) for ≥1 year, had a body mass index (BMI) >22.0 kg/m^2 and an HbA1c of 59 to 88 mmol/mol (7.5%–10.0%). The main exclusion criteria were insulin pump treatment, hypoglycaemia unawareness, diabetic gastroparesis, compromised kidney function and untreated proliferative retinopathy.[18] The Danish Medicines Agency (Eudract no. 2016-001365-92) and the Regional Scientific Ethics Committee of the Capital Region of Denmark (H-16034515) approved the trial, it was registered at the Danish Data Protection Agency and surveyed and guided by the Good Clinical Practice Unit for university hospitals associated with University of Copenhagen. All participants provided written informed consent.

2.2 | Randomization and blinding

Details about randomization and blinding were reported previously.[13] In short, a computer-generated randomization list (50 exenatide; 50 placebo) was made by a third party not involved in the trial and a further

eight slots (four exenatide; four placebo) were added during the trial because of a larger-than-expected dropout rate. Participants were consecutively allocated on a 1:1 ratio to 10 μg short-acting exenatide (Byetta®; AstraZeneca, Cambridge, UK) or placebo (an indistinguishable liquid placebo formulation) by two other persons not involved in the trial. Participants and study staff were masked to treatment allocation. During the trial, as participants were enrolled consecutively, two other individuals who were not involved in the trial consecutively allocated participants to treatment groups from the randomisationlist. The study drug package numbers were given to study staff who double-checked these with the actual packages. The packages were given to study participants. Both study staff and participants were masked totreatment allocation. All study drug pens and cartridgeswere indistinguishable. All statistical analyses were doneby individuals masked to treatment allocation.

2.3 | Procedures and measurements

Four visits were planned at weeks 0 (randomization), 4, 12 and 26 (end-of-treatment), with fasting from 10:00 PM the night before. Body composition was measured by whole-body dual-energy X-ray absorptiometry (DXA) scans at weeks 0 and 26 using a Hologic Discovery QDR series 82 800 Apex 3.3 scanner (Hologic Canada ULC, Mississauga, Canada). At weeks 0, 12 and 26, BMI, waist circumference, hip circumference and waist: hip ratio were measured by study staff. Fasting blood samples were drawn at weeks 0, 4, 12 and 26, and morning spot urine samples were collected at weeks 0, 12 and 26 for storage in a biobank at −80°C. From these stored samples, the following biomarkers were analysed after study completion: plasma levels of IL-2, IL-6 and TNF-α were analysed by multiplex sandwich electrochemiluminescence immunoassays using a V-PLEX Custom Human Cytokine kit (Meso Scale Diagnostics, Rockville, Maryland). CRP was quantified in plasma by a high-sensitivity latex-enhanced immunoturbidimetric assay for the Atellica CH 930 analyser (Siemens Healthcare Diagnostics Inc., Tarrytown, New York). NT-proBNP in plasma was analysed using a Roche NT-proBNP device (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). 8-oxodG and 8-oxoGuo were analysed in spot urine samples using ultra-performance liquid chromatography tandem mass spectrometry, as described elsewhere,[19] and adjusted to urine creatinine.

2.4 | Statistical analysis

All reported outcomes in the present study were prespecified secondary endpoints, except for 8-oxodG and 8-oxoGuo, which were added as secondary endpoints after study commencement but prior to trial end.[18] The sample size of the study population (n = 100) was based on a presumed 9mmol/mol (0.8%) standard deviation and enabled detection of an end-of-treatment difference between groups in HbA1c of 6 mmol/mol (0.5%) with 80% power and a 5% significance level. However, a bigger-than-expected dropout of 23 individuals (17 from the exenatide group, six from the placebo group) required an additional eight participants. Following study completion and analysis of arginine-stimulated serum C-peptide levels, participants with abnormally high C-peptide levels had their medical history and patient record closely reviewed. This resulted in two participants with exenatide (one was a dropout) and one participant with placebo being withdrawn from the statistical analysis due to reclassification of diagnosis to type 2 diabetes.[13] In total, 105 participants were included in the statistical analyses. Baseline continuous data are means with standard deviation, medians with minimum and maximum range or, for categorical variables, numbers with percentage. Continuous data not normally distributed were log-transformed. For efficacy analysis, we used a baseline-adjusted linear mixed model based on a likelihood ratio test, with visit, treatment and their interaction as fixed factors and a random effect on participant level in the intention-to-treat population.[20] Maximum likelihood estimation was used for missing data handling (equivalent to multiple imputation). As sensitivity analysis, all endpoints were compared with an unstructured covariance pattern. For the additional *post hoc* analysis of 8-oxodG and 8-oxoGuo, to avoid bias of weight changes during the study that may impact urine creatinine levels used to assess these biomarkers, we applied a model that estimates 24-hour excretion rates of 8-oxoGuo and 8-oxodG.[21] Also *post hoc*, we stratified participants at baseline into cardiovascular risk categories of very high risk (risk of cardiovascular death >10% within 10 years), high risk (risk of cardiovascular death 5%–10% within 10 years) and moderate risk (lower than high risk) as defined by the European Society of Cardiology.[22] In addition, *post hoc*, we used the "Steno Type 1 Diabetes Risk Engine" (https://steno.shinyapps.io/T1RiskEngine/) for a risk stratification that resembles clinical practice at the study site.[23] This risk stratification stratifies patients into high-risk (10-year cardiovascular risk ≥20%), moderate-risk (10%–20%) or low-risk categories (<10%). As we report only secondary endpoints, *P* values were not used for assessing statistical significance. However, all reported *P* values were adjusted for multiple testing.[24] R statistical software package (version 3.4.1) was used for all statistical analyses. Figures were designed using GRAPHPAD PRISM (version 8.0.2.263).

3 | RESULTS

3.1 | Study population

As previously described,[13] the included participants were, on average, middle-aged with longstanding type 1 diabetes, had moderate glycaemic dysregulation and were overweight, with total insulin requirements of 60 U/d and the majority were men (Table 1). Cardiovascular disease risk factors were evenly distributed between groups except for smoking, physical exercise level and diagnosis of simplex retinopathy (Table 1). When stratified into cardiovascular risk categories according to the European Society of Cardiology, in the exenatide group, 54% of participants were at very high risk, 21% at high risk and 25% at moderate risk. In the placebo group, 41% of participants were at very high risk, 19% at high risk and 40% at moderate risk (Table 1). For cardiovascular risk, as stratified by the "Steno Type 1 Diabetes Risk Engine", 40% of exenatide-treated participants were at high risk, 37% at moderate risk and 23% at low risk. With placebo treatment, 38% were at high risk, 38% at moderate risk and 25% at low risk (Table 1).

TABLE 1 Baseline information

	Exenatide (n = 52)	Placebo (n = 53)
Age, years	50.1 (14.2)	50.4 (14.0)
Men, n (%)	39 (75)	37 (70)
White ethnicity, n (%)	52 (100)	53 (100)
Diabetes duration, years	21.2 (11.3)	21.0 (12.9)
HbA1c, mmol/mol	66.8 (7.9)	65.9 (6.5)
HbA1c, %	8.3 (0.8)	8.2 (0.6)
Weight, kg	89.7 (14.4)	85.8 (14.3)
BMI, kg/m^2	29.0 (4.8)	27.7 (4.1)
Basal insulin requirement, U/d	31.2 (11.1)	29.7 (13.7)
Prandial insulin requirement, U/d	30.2 (13.7)	29.6 (19.6)
Systolic blood pressure, mmHg	129.0 (13.1)	129.0 (15.7)
Diastolic blood pressure, mmHg	82.0 (8.3)	82.5 (9.5)
Heart rate, beats/min	69.8 (10.4)	69.8 (11.3)
Total cholesterol, mmol/L	4.15 (0.87)	4.05 (0.89)
HDL cholesterol, mmol/L	1.37 (0.37)	1.34 (0.36)
LDL cholesterol, mmol/L	2.38 (0.73)	2.34 (0.83)
VLDL cholesterol, mmol/L	0.40 (0.19)	0.37 (0.19)
Triglycerides, mmol/L	0.87 (0.39)	0.83 (0.42)
eGFR, mL/min/1.73m^2	87.7 (5.8)	86.2 (7.7)
Cardiovascular risk category[a], n (%)		
Very high risk	28 (54)	22 (41)
High risk	11 (21)	10 (19)
Moderate risk	13 (25)	21 (40)
Cardiovascular risk category[b], n (%)		
High	21 (40)	20 (38)
Moderate	19 (37)	20 (38)
Low	12 (23)	13 (25)
Alcohol consumption, n (%)		
Yes	12 (23)	8 (15)
No	40 (77)	45 (85)
Smoking, n (%)		
Current	7 (13)	10 (19)
Never	32 (62)	25 (47)
Former	12 (23)	18 (34)
Exercise (>30 min), n (%)		
Daily	11 (21)	16 (30)
3–4 times weekly	9 (17)	14 (26)
1–2 times weekly	17 (33)	13 (25)
Rarely	12 (23)	8 (15)
Never	3 (6)	2 (4)
Comorbidities, n (%)		
Hypertension	23 (44)	21 (40)
1 class	12 (23)	11 (21)
2 classes	6 (12)	4 (8)
3 classes	2 (4)	4 (8)
>3 classes	3 (6)	2 (4)
Dyslipidaemia, n (%)	24 (46)	24 (45)
Cardiovascular disease, n (%)	2 (4)	6 (11)
Renal function, n (%)		
Normo-albuminuria: UACR >10 mg/g	48 (92)	47 (89)
Micro-albuminuria: UACR 30 to 300 mg/g	4 (8)	5 (9)
Macro-albuminuria: UACR >300 mg/g	0 (0)	1 (2)
Retinopathy		
Simplex	20 (38)	13 (25)
Proliferative	1 (2)	0 (0)
Peripheral neuropathy	15 (29)	11 (21)
Medication, n (%)		
Anti-hypertensive agent	23 (44)	21 (40)
Aspirin	7 (13)	9 (17)
Lipid-lowering agent	31 (60)	32 (60)

Data are mean (SD), unless otherwise indicated.
Abbreviations: 7-SMBG, seven-point self-monitoring of blood glucose; BMI, body mass index; CGM, continuous glucose monitoring; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; UACR, urinary albumin:creatinine ratio.
[a]Participants were stratified into cardiovascular risk categories as defined by the 2019 guidelines by European Society of Cardiology and European Association for the Study of Diabetes.
[b]Participants were stratified into cardiovascular risk categories as defined by the "Steno Type 1 Diabetes Risk Engine".

3.2 | Body composition

After 26 weeks, exenatide caused marked reductions in BMI, waist circumference and hip circumference (Table 2). Evaluated from DXA scans, exenatide changed total fat mass by −2.6 kg (95% CI −3.6 to −1.6; $P < 0.0001$) and lean + bone mineral content body mass by −1.1 kg (95% CI −1.9 to −0.4; $P = 0.01$) as compared with placebo (Figure 1). The fat mass loss was reflected in substantial reductions of total fat mass (%), fat mass index (kg/m^2), android fat mass and gynoid fat mass. Waist: hip ratio, measures of central and peripheral fat mass distribution and android/gynoid ratio were unchanged (Table 2).

3.3 | Biomarkers

8-oxoGuo, a marker of RNA oxidation, and 8-oxodG, a marker of DNA oxidation, both increased over time, and exenatide caused a slight elevation as compared with placebo at end-of-treatment. However, this between-group difference diminished when applying a model that

TABLE 2 Body composition

	Week 0	Week 12			Week 26		
	Pooled group Mean (95% CI)	Exenatide Mean (95% CI)	Placebo Mean (95% CI)	Mean ETD (95% CI); *P* value	Exenatide Mean (95% CI)	Placebo Mean (95% CI)	Mean ETD (95% CI); *P* value
BMI, kg/m^2	28.3 (27.5; 29.2)	27.4 (26.5; 28.2)	28.5 (27.6; 29.4)	−1.1 (−1.5; −0.8); <0.0001	26.9 (26.1; 27.8)	28.4 (27.5; 29.2)	−1.4 (−1.8; −1.1); <0.0001
Waist circumference, cm	96.9 (94.3; 99.5)	93.9 (90.9; 96.9)	97.7 (94.9; 100.6)	−3.8 (−6.4; −1.3); 0.01	94.1 (91.1; 97.1)	99.9 (97.0; 102.7)	−5.8 (−8.3; −3.2); <0.0001
Hip circumference, cm	104.5 (102.8; 106.2)	100.0 (97.9; 102.0)	103.3 (101.4; 105.3)	−3.4 (−5.2; −1.5); 0.01	100.8 (98.8; 102.9)	103.9 (102.0; 105.9)	−3.1 (−5.0; −1.2); 0.01
Waist: hip ratio	0.93 (0.91; 0.94)	0.94 (0.92; 0.96)	0.94 (0.92; 0.96)	0.00 (−0.03; 0.02); 0.90	0.94 (0.91; 0.96)	0.96 (0.94; 0.98)	−0.02 (−0.05; 0.00); 0.01
Total fat mass, kg	27.7 (25.9; 29.5)	–	–	–	25.2 (23.3; 27.1)	27.8 (25.9; 29.6)	−2.6 (−3.6; −1.6); <0.0001
Total fat mass, %	31.2 (29.6; 32.8)	–	–	–	29.7 (28.0; 31.4)	31.3 (29.7; 33.0)	−1.7 (−2.5; −0.9); <0.0001
Fat mass index, kg/m^2	9.5 (8.4; 10.6)	–	–	–	8.6 (7.5; 9.7)	9.5 (8.4; 10.6)	−0.9 (−1.2; −0.5); <0.0001
Trunk/limb fat mass ratio	1.01 (0.96; 1.06)	–	–	–	1.00 (0.94; 1.05)	1.01 (0.96; 1.07)	−0.02 (−0.05; 0.01); 0.35
Trunk fat percentage/leg fat percentage	0.97 (0.93; 1.00)	–	–	–	0.96 (0.92; 1.00)	0.96 (0.92; 1.01)	−0.01 (−0.03; 0.02); 0.54
Android fat mass, kg	2.6 (2.4; 2.9)	–	–	–	2.3 (2.1; 2.6)	2.6 (2.4; 2.9)	−0.3 (−0.4; −0.2); <0.0001
Gynoid fat mass, kg	4.7 (4.4; 5.0)	–	–	–	4.3 (3.9; 4.6)	4.7 (4.4; 5.0)	−0.4 (−0.6; −0.2); <0.0001
Android/gynoid ratio	1.06 (1.02; 1.10)	–	–	–	1.03 (0.98; 1.08)	1.05 (1.01; 1.10)	−0.02 (−0.05; 0.01); 0.31
Lean + BMC body mass (kg)	60.2 (58.2; 62.2)	–	–	–	58.8 (56.7; 60.9)	60.0 (57.9; 62.0)	−1.1 (−1.9; −0.4); 0.01

Abbreviations: BMC, bone mineral content; BMI, body mass index; ETD, estimated treatment difference. – indicates no data.

estimated 24-hour excretion of 8-oxoGuo and 8-oxodG instead of creatinine-adjusted values (Table 3). Beside this, no relevant relative median differences between groups were seen in end-of-treatment concentrations of IL-2, IL-6, TNF-α, hsCRP or NT-proBNP (Table 3). Of note, median hsCRP remained in the range of 1 to 2 mg/L at all time points. In addition, NT-proBNP stayed within normal range at all visits.

4 | DISCUSSION

In this randomized, double-blind, placebo-controlled clinical trial, testing the efficacy of short-acting exenatide administered three times daily before breakfast, lunch and dinner added to insulin therapy in type 1 diabetes and in a study population with overall high risk of cardiovascular disease, exenatide elicited relevant body weight reductions mediated by greater reductions of fat mass as compared with lean mass and with equal reductions in centrally and peripherally located fat mass. Biomarkers related to cardiovascular disease risk showed no between-group differences. Measures of RNA and DNA oxidation increased over 26 weeks, and exenatide increased both markers as compared with placebo at end-of-treatment, but this increase diminished when corrected for changes in body weight.

4.1 | Strengths and limitations

All reported outcomes were protocol-specified secondary endpoints except for 8-oxodG and 8-oxoGuo. As such, our findings cannot exclude an effect of short-acting exenatide on the distribution of central versus peripheral fat mass loss or plasma levels of IL-2, IL-6, TNF-α, hsCRP or

FIGURE 1 Efficacy of short-acting exenatide administered three times daily as compared with placebo in participants with type 1 diabetes. Change in mean body weight after 26 weeks stratified by fat mass and lean mass, as assessed by dual-energy X-ray absorptiometry scanning. Values are between-group mean differences with 95% confidence intervals or intra-group mean difference ± SEM from a linear mixed model incorporating baseline adjustment that handles missing data with maximum likelihood estimation (equal to multiple imputation) in the intention-to-treat population. ETD, estimated treatment difference

TABLE 3 Biomarkers

	Week 0	Week 4			Week 12			Week 26		
	Pooled group Mean (95% CI)	Exenatide Mean (95% CI)	Placebo Mean (95% CI)	Mean ETD (95% CI); *P* value	Exenatide Mean (95% CI)	Placebo Mean (95% CI)	Mean ETD (95% CI); *P* value	Exenatide Mean (95% CI)	Placebo Mean (95% CI)	Mean ETD (95% CI); *P* value
IL-2[a] pg/mL	0.15 (0.12; 0.19)	0.14 (0.11; 0.18)	0.14 (0.11; 0.19)	0.98 (0.69; 1.39); 0.96	0.11 (0.08; 0.15)	0.18 (0.14; 0.23)	0.63 (0.44; 0.91); 0.03	0.12 (0.09; 0.17)	0.14 (0.10; 0.18)	0.89 (0.60; 1.30); 0.54
IL-6[a] pg/mL	0.85 (0.76; 0.94)	0.93 (0.81; 1.07)	1.01 (0.88; 1.16)	0.92 (0.78; 1.09); 0.54	0.97 (0.83; 1.12)	0.99 (0.86; 1.13)	0.98 (0.82; 1.17); 0.86	0.84 (0.72; 0.97)	0.91 (0.79; 1.05)	0.92 (0.77; 1.10); 0.40
TNF-α[a] pg/mL	1.97 (1.86; 2.07)	2.01 (1.89; 2.14)	2.09 (1.96; 2.22)	0.96 (0.90; 1.02); 0.51	2.03 (1.91; 2.17)	2.16 (2.03; 2.30)	0.94 (0.88; 1.00); 0.12	1.86 (1.74; 1.98)	1.95 (1.83; 2.07)	0.95 (0.89; 1.02); 0.21
hsCRP[a], mg/L	1.62 (1.32; 2.00)	1.21 (0.94; 1.57)	1.61 (1.25; 2.06)	0.76 (0.57; 1.00); 0.23	1.44 (1.11; 1.89)	1.87 (1.46; 2.41)	0.77 (0.58; 1.03); 0.13	1.42 (1.08; 1.86)	1.69 (1.31; 2.18)	0.84 (0.62; 1.13); 0.31
NT-proBNP[a], pmol/L	7.80 (7.12; 8.54)	7.38 (6.62; 8.23)	7.36 (6.62; 8.19)	1.00 (0.90; 1.12); 0.97	7.88 (7.03; 8.83)	7.69 (6.90; 8.56)	1.02 (0.91; 1.15); 0.80	7.48 (6.67; 8.40)	7.08 (6.36; 7.89)	1.06 (0.94; 1.19); 0.40
8-oxoGuo/creatinine, nmol/mmol	1.69 (1.59; 1.78)	–	–	–	1.83 (1.70; 1.97)	1.77 (1.65; 1.90)	0.06 (−0.10; 0.22); 0.69	2.20 (2.06; 2.33)	2.03 (1.91; 2.15)	0.16 (0.00; 0.33); 0.09
8-oxodG/creatinine, nmol/mmol	1.26 (1.17; 1.35)	–	–	–	1.46 (1.35; 1.58)	1.25 (1.14; 1.36)	0.21 (0.08; 0.34); 0.01	1.60 (1.48; 1.72)	1.46 (1.35; 1.57)	0.14 (0.01; 0.27); 0.08
24-hour 8-oxoGuo, nmol/24 h	17.78 (16.65; 18.91)	–	–	–	19.24 (17.65; 20.84)	18.71 (17.24; 20.18)	0.53 (−1.36; 2.42); 0.76	22.94 (21.32; 24.57)	21.46 (20.00; 22.91)	1.49 (−0.42; 3.39); 0.20
24-hour 8-oxodG, nmol/24 h	13.33 (12.29; 14.37)	–	–	–	15.51 (14.17; 16.84)	13.25 (12.00; 14.50)	2.25 (0.85; 3.65); 0.01	16.71 (15.36; 18.06)	15.47 (14.23; 16.71)	1.24 (−0.17; 2.65); 0.14

Data are means (95% confidence interval [CI]), mean differences (95% CI) with *P values*, or [a]medians with 95% confidence intervals in brackets or [a]relative difference of medians (95% CI) with *P values*.
Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; ETD, estimated treatment difference; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; TNF, tumour necrosis factor.
– indicates no data.

NT-proBNP. However, considering the 95% CIs of these endpoints, an effect of exenatide seems unlikely. The larger-than-expected differential dropout in the exenatide treatment arm may cause bias; however, our handling of missing data took this into account. To elaborate, maximum likelihood estimation (equivalent to multiple imputation) inherent in the statistical model imputed missing data for dropouts according to their designated treatment group. For further details of our handling of missing data in the MAG1C trial, see the supplementary material for the publication in which the primary results were reported.[13] Also, short-acting exenatide has a half-life of 2.4 hours and, thus, fluctuating plasma levels over the day could attenuate its impact on risk of cardiovascular disease as compared with more stable plasma levels of long-acting GLP-1RAs. Our study population of glycaemic dysregulated, normal-to-overweight/obese patients with longstanding type 1 diabetes represented an overall high-risk patient group of cardiovascular disease as assessed by two different cardiovascular risk stratification algorithms. However, a discrepancy between baseline risk of cardiovascular disease, as defined by the European Society of Cardiology,[22] should be noted as opposed to a similar distribution of risk of cardiovascular disease as defined by "Steno Type 1 Diabetes Risk Engine".[23] We speculate this to be a chance finding as the majority of baseline covariates were evenly distributed between groups. Notably, our study population complicates generalizability as it consisted of Danish patients of white ethnicity, and male sex was overrepresented. Finally, 26 weeks is a short period in which to conclude on possible changes in cardiovascular disease risk and, as the assessed endpoints are indirect markers of cardiovascular disease, it should be stressed that the reported findings should be interpreted with caution.

4.2 | Body composition

As evaluated by DXA scans, after 26 weeks, exenatide caused greater reductions in total fat mass as compared with total lean mass, whereas measures of central and peripheral fat mass loss were unchanged. Exenatide caused marked reductions of BMI, waist circumference and hip circumference but did not affect waist: hip ratio. To our knowledge, this is the first time the impact of GLP-1RA treatment on body composition has been assessed by DXA in type 1 diabetes. A small (n = 15) crossover study evaluating once-daily 1.8 mg liraglutide over 24 weeks as add-on treatment to insulin therapy in obese individuals reported reductions of fat mass of 3.8 kg and fat-free mass of 1.0 kg as assessed by bioimpedance analysis; reductions in BMI, waist circumference and hip circumference were similar to ours.[25] Also, 4 weeks' treatment with short-acting exenatide or the short-acting GLP-1RA, lixisenatide, in small, mainly mechanistic studies reported BMI reductions of 0.5 to 1.0 kg/m^2 as compared with placebo.[26–28] Likewise, in a 12-week randomized, double-blind, placebo-controlled clinical trial (n = 40), 1.2 mg liraglutide once daily reduced BMI by 1.0 kg/m^2.[10] However, a 5kg/m^2 lower baseline BMI compared to our trial and only 12 weeks' intervention complicate comparisons. Taken together, our body composition results suggest that short-acting exenatide induces primarily fat-mass loss, but any particular effect on central obesity is unclear from the present data; possibly due to insufficient sample size. Notably, short-acting

exenatide seems to improve insulin sensitivity as assessed by the hyperinsulinaemic-euglycaemic clamp technique.[29]

4.3 | Biomarkers

In our study, exenatide elicited no changes in proinflammatory cytokines, TNF-α and IL-6, or acute phase reactant, CRP, after 26 weeks as compared with placebo. Interestingly, infusion of GLP-1 attenuated the rise of IL-6 during a hypoglycaemic clamp and a hyperglycaemic clamp as compared with placebo.[30] In relation to these biomarkers and cardiovascular disease risk, a large, cross-sectional case–control study in type 1 diabetes (n = 543) reported that increased mean levels of CRP, IL-6 and TNF-α (in ranges comparable with our reported median values) were all strongly associated with the presence of cardiovascular disease in a crude model. When adjusted for age, sex, HbA1c, diabetes duration and systolic blood pressure, only CRP lost its association with cardiovascular disease.[31] Also in a case–control study (n = 136), increased levels of a compound low-grade inflammation score (IL-6, CRP and soluble receptors 1 and 2 of TNF-α) were independently associated with arterial stiffness, a marker of cardiovascular disease, in men but not women with type 1 diabetes.[32] Taken together, our median levels of IL-6, TNF-α and CRP remained constant during trial; and our median levels compared with reported means in other studies; and exenatide did not change any of these biomarkers as compared with placebo. Exenatide's lack of effect on IL-6, TNF-α and CRP could be attributable to an overall low-grade inflammation status, as suggested by the median CRP levels in the average risk range of cardiovascular disease of 1–3 mg/L.[33]

Exenatide did not change relative median IL-2 levels as compared with placebo after 26 weeks in the present study. Interestingly, IL-2 stimulates regulatory T-cell activity, and reduced levels of regulatory T cells have recently been correlated with cardiovascular disease in type 1 diabetes.[34] In contrast, elevated IL-2 levels have been linked to reduced progression of atherosclerosis in mouse models.[35] We speculate that exenatide's lack of effect on IL-2 plasma levels could be attributable to the low inflammation levels as indicated by TNF-α, IL-6 and CRP in the study population.

Plasma levels of NT-proBNP were stable during the study period, and exenatide elicited no changes as compared with placebo. Importantly, increased levels of NT-proBNP, as a marker of left ventricular dysfunction and, indirectly, coronary heart disease, have been found to correlate with cardiovascular disease in type 1 diabetes.[36,37] Notably, our reported median levels were < 8 pmol/L (within normal range), limiting detection of an impact of short-acting exenatide on cardiovascular disease risk.

Oxidatively generated DNA and RNA modifications measured as urinary excretion of 8-oxodG and 8-oxoGuo increased from baseline until end-of-treatment. Exenatide increased both markers as compared with placebo after 26 weeks, but these between-group differences diminished in a model accounting for body weight changes. Interestingly, in type 2 diabetes, increased levels of 8-oxoGuo have been associated with increased risk of cardiovascular death,[38] and GLP-1RA-mediated reductions of cardiovascular risk have been suggested to be partly mediated by reductions of oxidative stress.[39] However, our results do not indicate a beneficial effect of short-acting exenatide on oxidative stress in patients with type 1 diabetes.

4.4 | Previously reported endpoints related to cardiovascular disease

Ambulatory blood pressure and heart rate, together with blood lipid levels, were previously reported but are of interest to the present discussion of short-acting exenatide's impact on risk of cardiovascular disease in type 1 diabetes. Exenatide increased heart rate by 3.9 beats per minute (95% CI 0.5 to 7.2; $P = 0.16$) as compared with placebo after 26 weeks' treatment. Exenatide did not change ambulatory blood pressure or blood lipid levels at end-of-treatment as compared with placebo.[13]

4.5 | Conclusions

Taken together, short-acting exenatide administered three times daily before breakfast, lunch and dinner as add-on treatment to multiple daily injection therapy in type 1 diabetes in a study population with overall high risk of cardiovascular disease resulted in clinically relevant reductions in body weight, primarily derived from a reduction of both central and peripheral fat mass. Exenatide did not affect low-grade inflammatory status as assessed by IL-2, IL-6, TNF-α and CRP, nor cardiac function as assessed by NT-proBNP. Finally, exenatide did not markedly impact DNA/RNA oxidation status assessed by 8-oxodG and 8-oxoGuo, respectively. To our knowledge, no previous study has investigated the effects of GLP-1RA treatment on any of these biomarkers related to cardiovascular disease in type 1 diabetes.

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

N.J.J., T.F.D., A.L., T.V., H.U.A. and F.K.K. initiated and designed the trial. N.J.J., T.F.D., C.S., E.L.L., H.E.P., J.P.G., H.J.M. and H.U.A. participated in the data collection. N.J.J. performed the statistical analysis and wrote the first draft of the manuscript. All authors revised the manuscript for crucial intellectual content. N.J.J. and F.K.K. are the guarantors of this work and, as such, had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. Livingstone SJ, Levin D, Looker HC, et al. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. *JAMA*. 2015;6:37-44.
2. Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*. 1993;30:977-986.
3. Nathan DM, Cleary PA, Backlund J-YC, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med*. 2005;353:2643-2653.
4. Conway B, Miller RG, Costacou T, et al. Temporal patterns in overweight and obesity in Type 1 diabetes. *Diabet. Med*. 2010;27:398-404.
5. Purnell JQ, Zinman B, Brunzell JD, DCCT/EDIC Research Group. The effect of excess weight gain with intensive diabetes mellitus treatment on cardiovascular disease risk factors and atherosclerosis in type 1 diabetes mellitus: results from the diabetes control and complications trial/epidemiology of diabetes interventions and complications study (DCCT/EDIC) study. *Circulation*. 2013;127:180-187.
6. Kilpatrick ES, Rigby AS, Atkin SL. Insulin resistance, the metabolic syndrome, and complication risk in type 1 diabetes: "double diabetes" in the diabetes control and complications trial. *Diabetes Care*. 2007;30:707-712.
7. Orchard TJ, Olson JC, Erbey JR, et al. Insulin resistance-related factors, but not glycemia, predict coronary artery disease in type 1 diabetes: 10-year follow-up data from the Pittsburgh epidemiology of diabetes complications study. *Diabetes Care*. 2003;26:1374-1379.
8. McKnight JA, Wild SH, Lamb MJE, et al. Glycaemic control of Type 1 diabetes in clinical practice early in the 21st century: an international comparison. *Diabet. Med*. 2015;32:1036-1050.
9. Dejgaard TF, Frandsen CS, Hansen TS, et al. Efficacy and safety of liraglutide for overweight adult patients with type 1 diabetes and insufficient glycaemic control (Lira-1): a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol*. 2016;4:221-232.
10. Frandsen CS, Dejgaard TF, Holst JJ, Andersen HU, Thorsteinsson B, Madsbad S. Twelve-week treatment with liraglutide as add-on to insulin in normal-weight patients with poorly controlled type 1 diabetes: a randomized, placebo-controlled, double-blind parallel study. *Diabetes Care*. 2015;38:2250-2257.
11. Mathieu C, Zinman B, Hemmingsson JU, et al. Efficacy and safety of liraglutide added to insulin treatment in type 1 diabetes: the ADJUNCT ONE treat-to-target randomized trial. *Diabetes Care*. 2016;39:1702-1710.
12. Ahrén B, Hirsch IB, Pieber TR, et al. Efficacy and safety of liraglutide added to capped insulin treatment in subjects with type 1 diabetes: the ADJUNCT TWO randomized trial. *Diabetes Care*. 2016;39:1693-1701.
13. Johansen NJ, Dejgaard TF, Lund A, et al. Efficacy and safety of meal-time administration of short-acting exenatide for glycaemic control in type 1 diabetes (MAG1C): a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol*. 2020;8(4):313–324.
14. Frandsen CS, Dejgaard TF, Madsbad S. Non-insulin drugs to treat hyperglycaemia in type 1 diabetes mellitus. *Lancet Diabetes Endocrinol*. 2016;4:766-780.
15. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2016;375:311-322.
16. Marso SP, Bain SC, Consoli A, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2016;375:1834-1844.
17. Gerstein HC, Colhoun HM, Dagenais GR, et al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. *Lancet*. 2019;394:121-130.
18. Johansen NJ, Dejgaard TF, Lund A, Vilsbøll T, Andersen HU, Knop FK. Protocol for meal-time administration of exenatide for glycaemic control in type 1 diabetes cases (the MAG1C trial): a randomised, double-blinded, placebo-controlled trial. *BMJ Open*. 2018;8:e021861.
19. Rasmussen ST, Andersen JT, Nielsen TK, et al. Simvastatin and oxidative stress in humans: a randomized, double-blinded, placebo-controlled clinical trial. *Redox Biol*. 2016;9:32-38.
20. Fitzmaurice GM, Laird NM, Ware JH. *Applied Longitudinal Analysis*. 2nd ed. Hoboken, Canada: John Wiley & Sons; 2011.
21. Poulsen HE, Weimann A, Henriksen T, et al. Oxidatively generated modifications to nucleic acids in vivo: measurement in urine and plasma. *Free Radic Biol Med*. 2019;145:336-341.
22. Cosentino F, Grant PJ, Aboyans V, et al. 2019 ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J*. 2019;41:255-323.
23. Vistisen D, Andersen GS, Hansen CS, et al. Prediction of first cardiovascular disease event in type 1 diabetes mellitus. *Circulation*. 2016;133:1058-1066.
24. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*. 1995;57:289-300.
25. Dubé M-C, D'Amours M, Weisnagel SJ. Beyond glycaemic control: a cross-over, double-blinded, 24-week intervention with liraglutide in type 1 diabetes. *Diabetes Obes Metab*. 2018;20:178-184.
26. Jiang L-L, Wang S-Q, Ding B, et al. The effects of add-on exenatide to insulin on glycemic variability and hypoglycemia in patients with type 1 diabetes mellitus. *J Endocrinol Invest*. 2018;41:539-547.
27. van Meijel LA, Rooijackers HM, Tack CJ, de Galan BE. Effect of the GLP-1 receptor agonist exenatide on impaired awareness of hypoglycemia in type 1 diabetes; a randomized controlled trial. *J Clin Endocrinol Metab*. 2019;104:4143-4150.
28. Ballav C, Dhere A, Agbaje O, Kennedy I, Holman RR, Owen KR. The effect of lixisenatide on post-prandial blood glucose and glucagon in type 1 diabetes. *In Proceedings of the 54th EASD Annual Meeting of the European Association for the Study of Diabetes*. Berlin, Germany: Diabetologia; 2019;60(6):347-351.
29. Sarkar G, Alattar M, Brown RJ, Quon MJ, Harlan DM, Rother KI. Exenatide treatment for 6 months improves insulin sensitivity in adults with type 1 diabetes. *Diabetes Care*. 2014;37:666-670.

30. Ceriello A, Novials A, Ortega E, et al. Glucagon-like peptide 1 reduces endothelial dysfunction, inflammation, and oxidative stress induced by both hyperglycemia and hypoglycemia in type 1 diabetes. *Diabetes Care*. 2013;36:2346-2350.
31. Schram MT, Chaturvedi N, Schalkwijk CG, Fuller JH, Stehouwer CDA, EURODIAB Prospective Complications Study Group. Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes–the EURODIAB prospective complications study. *Diabetologia*. 2005;48:370-378.
32. Llauradó G, Ceperuelo-Mallafré V, Vilardell C, et al. Arterial stiffness is increased in patients with type 1 diabetes without cardiovascular disease: a potential role of low-grade inflammation. *Diabetes Care*. 2012;35:1083-1089.
33. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease. *Circulation*. 2003;107:499-511.
34. El-Shabrawy RM, Ahmed AM, Selim FO, Said NM. Association between CD4+, CD25+, FOXP3+ regulatory T-cells and cardiovascular complications in diabetic patients type 1. *Egypt J Immunol*. 2019;26:129-139.
35. Dinh TN, Kyaw TS, Kanellakis P, et al. Cytokine therapy with interleukin-2/anti-interleukin-2 monoclonal antibody complexes expands CD4+CD25+Foxp3+ regulatory T cells and attenuates development and progression of atherosclerosis. *Circulation*. 2012;126:1256-1266.
36. Grauslund J, Nybo M, Green A, Sjølie AK. N-terminal pro brain natriuretic peptide reflects long-term complications in type 1 diabetes. *Scand J Clin Lab Invest*. 2010;70:392-398.
37. Konduracka E, Cieslik G, Galicka-Latala D, et al. Myocardial dysfunction and chronic heart failure in patients with long-lasting type 1 diabetes: a 7-year prospective cohort study. *Acta Diabetol*. 2013;50:597-606.
38. Kjær LK, Cejvanovic V, Henriksen T, et al. Cardiovascular and all-cause mortality risk associated with urinary excretion of 8-oxoGuo, a biomarker for RNA oxidation, in patients with type 2 diabetes: a prospective cohort study. *Diabetes Care*. 2017;40:1771-1778.
39. Andrikou E, Tsioufis C, Andrikou I, Leontsinis I, Tousoulis D, Papanas N. GLP-1 receptor agonists and cardiovascular outcome trials: an update. *Hellenic J Cardiol*. 2019;S1109-60(6):347-351.

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