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Burggraaf, B.; Pouw, N.M.C.; Arroyo, S.F.; Vark-van der Zee, L.C. van; Geijn, G.J.M. van de; Birnie, E.; ... ; Cabezas, M.C.

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Benjamin Burggraaf[1], Nadine M C Pouw[2], Salvador Fernández Arroyo[3], Leonie C van Vark-van der Zee[4], Gert-Jan M van de Geijn[2], Erwin Birnie[5,7], Jeannine Huisbrink[6], Ellen M van der Zwan[2], Wouter W de Herder[8], Monique T Mulder[4], Patrick C N Rensen[9] and **Manuel Castro Cabezas[1,8]**

[1]Department of Internal Medicine, Center for Endocrinology, Diabetes and Vascular Medicine, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands, [2]Department of Clinical Chemistry, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands, [3]Departament de Medicina i Cirurgia, Unitat de Recerca Biomèdica, Universitat Rovira i Virgili, Tarragona, Spain, [4]Division of Pharmacology, Vascular and Metabolic Diseases, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands, [5]Department of Statistics and Education, Franciscus Academy, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands, [6]Department of Pharmacy, Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands, [7]Department of Genetics, University Medical Center Groningen, Groningen University, Groningen, the Netherlands, [8]Department of Internal Medicine, Section of Endocrinology, Erasmus Medical Center, Rotterdam, the Netherlands, and [9]Division of Endocrinology, Department of Internal Medicine, Leiden University Medical Center, Leiden, the Netherlands

Correspondence should be addressed to B Burggraaf
Email burggraaf.benjamin@gmail.com

Abstract

Objectives: Sodium-glucose cotransporter 2 inhibitors (SGLT2i) modulate lipid metabolism and improve cardiovascular morbidity and mortality in patients with type 2 diabetes mellitus (T2DM). The exact cardioprotective mechanism of SGLT2i is unclear. We evaluated the effects of SGLT2i on postprandial lipids, lipoprotein concentrations, glucose and fatty acids.

Design: A placebo-controlled randomized, proof-of-concept study.

Methods: Fourteen male patients with T2DM on intensive insulin regimen were randomly and double-blind allocated to 12 weeks dapagliflozin (10 mg) or placebo. Postprandial effects were assessed with an 8-h standardized oral fat loading test.

Results: Mean glycated A1c did not change by dapagliflozin, but the mean daily insulin dose was significantly reduced. Although dapagliflozin did not affect fasting or postprandial levels of glucose and insulin, it increased the postprandial levels of glucagon. While fasting levels of free fatty acids and beta-hydroxybutyrate (bHBA) were unchanged, dapagliflozin significantly increased the postprandial bHBA response. This was seen in the context of increased postprandial glucagon levels by dapagliflozin, without influencing postprandial insulin or glucose levels. Dapagliflozin did not affect fasting or postprandial plasma cholesterol and triglycerides nor postprandial inflammatory markers. Fasting apolipoprotein B48 was decreased without affecting the postprandial response. Markers of inflammation and vascular function did not change.

Conclusion: Treatment with dapagliflozin of patients with T2DM led to a reduction of fasting chylomicron remnants and increased postprandial ketone bodies compared to placebo suggesting enhanced hepatic fatty acid oxidation. The latter may have been caused by decreasing the insulin–glucagon ratio. The beneficial clinical effects seen in the trials using dapagliflozin most likely are not due to effects on postprandial inflammation nor postprandial lipemia.

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Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent diseases in the general population, leading to an increased risk of cardiovascular disease (1, 2, 3). While increased glucose levels have been associated with this increased cardiovascular disease risk (4), many other cardiovascular risk factors associated with T2DM, like atherogenic dyslipidemia and systemic inflammation, play an important role (5). This atherogenic dyslipidemia in T2DM includes the presence of small dense LDL-C, low HDL-C and postprandial dyslipidemia in which insulin resistance leads to an increased postprandial chylomicron production and reduced remnant clearance resulting in the accumulation of atherogenic chylomicron remnants (6).

Until recently, most studies aiming to improve blood glucose levels had not shown clinically significant reductions in cardiovascular mortality (6, 7). Sodium-glucose cotransporter 2 inhibitors (SGLT2i) have been identified as a new class of glucose-lowering drugs with beneficial cardiovascular effects. Due to enhanced urinary glucose secretion, SGLT2i lead to an insulin-independent decrease of blood glucose (8), reduction of postprandial insulin levels and elevation of postprandial glucagon levels (9). Cardiovascular outcome trials have shown that SGLT2i reduce cardiovascular mortality in high-risk patients with T2DM (10, 11, 12), although the most relevant clinical effects have been shown in patients with established cardiovascular disease (13). In those large clinical trials, the reduction of glycated A1c (HbA1c) was limited as predefined in the study designs. Therefore, SGLT2i likely modulate other cardiovascular disease risk factors, contributing to the observed reduced cardiovascular morbidity and mortality. Several underlying mechanisms have been proposed, including increased natriuresis leading to reduced myocardial stress, reduced blood pressure, renal hemodynamic effects, increased ketone bodies that act as a myocardial energy source and metabolic and anti-inflammatory effects (14).

In this proof-of-concept study, we aimed to evaluate the effects of the SGLT2i dapagliflozin on fasting and postprandial lipid and lipoprotein metabolism, glycemic factors and metabolic markers in addition to postprandial inflammatory markers and vascular function in patients with T2DM.

Methods

Study design and subjects

This study was designed as a single-center, randomized, double-blind, placebo-controlled, proof-of-concept study aimed at comparing dapagliflozin vs placebo on postprandial lipemia, inflammation and metabolic changes. All patients provided their written informed consent prior to any study-specific procedure. The study was approved by the Institutional Review Board of the Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands, the regional independent medical research ethics committee TWOR, Rotterdam and by the national competent authority. The study was registered at www.trialregister.nl under clinical trial no. NTR6709 and was conducted in accordance with the declaration of Helsinki.

Patients were recruited between September 2017 and September 2018 from the Diabetes and Vascular Center of the Franciscus Gasthuis & Vlietland. All consecutive patients with the required characteristics were screened for eligibility. Inclusion criteria were male sex, age >18 years old, established T2DM on intensive insulin therapy (daily one-time long-acting insulin and three times short-acting insulin, with a stable dosage or 10 weeks) and stable glucose regulation in the previous 6 months (HbA1c between 6.5 and 9.0%). Exclusion criteria were current smoking, decreased kidney function (estimated glomerular filtration rate <60 mL/min/1.73 m^2), a cardiovascular or hypoglycemic event in the previous 6 months and the use of an SGLT2i in the previous 6 months.

Data collection

All subjects visited the hospital after a 10-h overnight fast. Anthropomorphic characteristics (height, weight, BMI and waist circumference) and blood pressure were measured. The medical and family history were recorded. A fasting venous blood sample was obtained and baseline arterial vascular function was measured after 5 min of rest. Subjects received an oral fat load consisting of fresh cream (Albert Heijn, Zaandam, the Netherlands) in a dose of 50 g of fat/m^2 body surface. During the oral fat loading test, participants were not allowed to eat or to drink except water and they were asked to refrain from physical activity. Venous blood sampling and arterial vascular function measurements were repeated every 2 h until 8 h after ingestion of the cream.

Outcome measures

The primary endpoint of this study was the change from baseline of postprandial apolipoprotein (apo) B48 after treatment with dapagliflozin compared to placebo. Secondary endpoints were the changes from baseline of

postprandial triglycerides, glucose, insulin and glucagon. Additional endpoints were fasting and postprandial levels of free fatty acids (FFA), glucagon, beta-hydroxybutyrate (bHBA), leukocyte activation markers and vascular function.

Randomization, blinding and treatment

After the first oral fat loading test, patients were randomized 1:1 to receive dapagliflozin 10 mg or matching placebo once daily for 12 weeks (both kindly provided by AstraZeneca). Randomization was based on computer-generated block randomization. Both physicians and subjects were blinded to the allocated treatment.

Patients were instructed to take dapagliflozin or placebo daily in the morning on top of their previous standard glucose-lowering regimen. Patients were allowed to down titrate their insulin dosage to avoid hypoglycemia. After 12 weeks of treatment, the participants visited the outpatient clinic for the second oral fat loading test. Compliance to study medication was evaluated by counting pills remaining in the dispenser. Information on the occurrence and type of side effects was also recorded.

Vascular function

Carotid to femoral pulse wave velocity (PWV) and augmentation index (Aix) were measured and calculated with the SphygmoCor Electronics Module MM3 and SphygmoCor CvMS Software Suite version 8.0 (AtCor Medical, West Ryde, Australia). The PWV, which increases at higher aortic stiffness, was measured using a noninvasive tonometry at the carotid and femoral artery. The distance between the carotid and femoral artery was measured with a measuring tape. The Aix, adjusted to a heart rate of 75 b.p.m., represents both macrovascular and microvascular functions. The radial artery was used to measure the Aix. Both, the PWV and Aix were measured in duplicate at each time interval and the mean value is reported.

Laboratory measurements

All clinical chemistry and hematology measurements were carried out on freshly drawn blood at the department of Clinical Chemistry, Franciscus Gasthuis & Vlietland according to standard procedures, unless otherwise stated. Renal and liver function as well as C-reactive protein, glucose, total plasma cholesterol, HDL-C and triglycerides were measured in plasma using an Architect c8000 (Abbott). Plasma LDL-C values were calculated using the Friedewald formula for TG below 4.0 mmol/L. For TG values >4.0 mmol/L, no LDL-C levels could be calculated and therefore no postprandial LDL-C levels are reported. ApoA-I and apoB were determined in serum by nephelometry using an IMMAGE instrument with commercially available kits (Beckman Coulter, Miami, USA). Blood cell counts were determined in plasma samples using a DxH analyzer (Beckman Coulter). HbA1c was measured in plasma samples with a G8 analyzer (Tosoh, San Francisco, CA, USA).

ApoB48 serum levels, the marker for intestinal chylomicron particles, were quantified using a commercially available enzyme immune assay (ELISA) (Shibayagi Co., Ltd. Japan), according to the manufacturer's instructions (15). Since no commercial quality controls are available for apoB48, a local internal quality control was pooled according to WHO recommendations, stored at −80°C, and assessed in duplicate on each plate in parallel to samples. Paired samples were measured in the same run.

Serum FFA were measured by an enzymatic colorimetric assay (WAKO kit, Fujifilm WAKO Diagnostics) and plasma glucagon was measured by a RIA (Merck) at the division of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands. Serum bHBA levels were measured by photometrical analyses using a Cobas analyzer (Roche) at the Department of Internal Medicine, Division of Pharmacology, Vascular and Metabolic Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands. Serum insulin was measured by an immune radiometric assay at the department of Clinical Chemistry, IJsselland Ziekenhuis, Cappelle a/d IJssel, the Netherlands.

Leukocyte activation markers were measured in plasma as described in detail previously (16, 17). Briefly, the staining procedure was started within 30 min after venipuncture. All measurements were carried out in triplicate and mean values are reported. Whole blood was added to a combination of fluorescein isothiocyanate (FITC)-conjugated CD66b, phycoerythrin cyanin (PC5)-conjugated CD11b, phycoerythrin (PE)-conjugated CD35 and phycoerythrin-Texas Red-X (ECD)-conjugated CD45. In parallel, blood was added to a combination of FITC, PC5- and PE-conjugated mouse IgG1 as isotype controls to correct for non-specific binding. All antibodies were from Beckman Coulter, except for CD35-PE (BD Bioscience). After incubation for 15 min in the dark at room temperature, erythrocytes were lysed by adding isotonic erythrocyte lysing solution (0.19 M ammonium chloride, 0.01 M potassium hydrogen carbonate, 0.12 M EDTA, pH

7.2) for 15 min. An FC500 flow cytometer and Kaluza 1.5a software (Beckman Coulter) were used for measurement and analysis. Lymphocytes, monocytes and neutrophil granulocytes were identified based on their side scatter and the level of CD45 on their surface. The fluorescent intensity of each cell population was expressed as the mean fluorescent intensity.

Statistical analysis

Data are given as mean ± S.D. for normally distributed variables, median (interquartile range) for variables with skewed distribution, and number (%) for categorical variables. In all figures, data are presented as mean ± S.E.M.

The main treatment effect (p-model) of dapagliflozin vs placebo on outcome values between pre- and post-treatment levels was tested with ANCOVA with the post-treatment levels as dependent variable, pretreatment levels and diabetes duration as covariates and treatment group as a fixed effect. Skewed data were logarithmically transformed; however, unchanged data are presented. Data are presented as a change in percentage of outcome compared to baseline by dapagliflozin vs placebo.

Differences within groups before and after treatment (for both the fasting and postprandial levels) were determined by the paired Student's *t*-test. Differences between groups were determined by the independent Student's *t*-test or Mann–Whitney *U* test, where appropriate. All postprandial responses were calculated using the total area under the curve by the trapezoidal rule using Graphpad Prism version 5.0.

Statistical analysis was carried out with PASW statistics version 25.0 (IBM SPSS Statistics). *P*-values <0.05 (two-tailed) were considered statistically significant.

Results

Baseline patient characteristics

Fourteen eligible patients were included and randomized to either dapagliflozin ($n = 8$) or placebo ($n = 6$). All patients were on insulin regimen four times daily, with an average dose of 70.8 ± 29.3 units per day. All patients were using metformin; no other antidiabetic agents were used. All patients were treated with statins, which were unaltered during the study, but no other hypolipidemic agents were used. Additional characteristics are shown in Table 1 for the total cohort and per treatment group. During the study, no serious adverse events were recorded for either group.

Effects on fasting parameters

Table 2 shows the effect of treatment with dapagliflozin on fasting levels of all analyzed parameters. Twelve weeks of treatment with dapagliflozin resulted in a significantly decreased daily dose of insulin (median change −14.8%; p-model = 0.03), fasting apoB48 (−39.8%; p-model = 0.01), and systolic blood pressure (−1.9%; p-model = 0.04) compared to treatment with placebo. Dapagliflozin did not significantly affect glycated A1c (+1.7%; p-model = 0.77) or fasting glucose levels (−9.8%; p-model = 0.39), fasting plasma triglycerides (+9.4%;

Table 1 Baseline characteristics of study participants ($n = 14$). Data as mean ± S.D., or median (interquartile range).

	Total ($n = 14$)	**Dapagliflozin** ($n = 8$)	**Placebo** ($n = 6$)
Age (years)	64.6 ± 8.6	64.9 ± 8.0	64.2 ± 10.2
Body mass index (kg/m^2)	29.4 ± 3.9	29.4 ± 2.9	29.4 ± 5.2
Waist circumference (cm)	104.9 ± 6.0	104.9 ± 6.2	104.8 ± 6.3
Systolic blood pressure (mmHg)	140.1 ± 16.4	134.0 ± 9.9	148.2 ± 20.6
Fasting glucose (mmol/L)	9.7 ± 3.1	9.6 ± 3.0	9.8 ± 3.4
Glycated A1c (mmol/mol)	66.0 ± 12.1	66.6 ± 10.7	65.2 ± 14.9
Total cholesterol (mmol/L)	3.8 ± 0.7	3.9 ± 0.7	3.6 ± 0.7
HDL-cholesterol (mmol/L)	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.2
LDL-cholesterol (mmol/L)	2.2 ± 0.6	2.5 ± 0.6	1.9 ± 0.6
Triglycerides (mmol/L)	1.22 (0.87–1.57)	1.13 (0.84–1.42)	1.33 (0.75–1.91)
ApoAI (g/L)	1.36 ± 0.60	1.27 ± 0.18	1.30 ± 0.23
ApoB (g/L)	0.78 ± 0.23	0.81 ± 0.15	0.72 ± 0.17
ApoB48 (mg/L)	10.2 (5.5–14.9)	11.8 (8.6–14.9)	9.3 (3.6–14.9)
Lipoprotein(a) (mg/L)	130.5 (0.0–386.5)	138.5 (0.0–498.5)	117.6 (0.0–314.5)
Diabetes duration (years)	17.6 ± 8.6	13.5 ± 7.4	23.2 ± 7.3*
Insulin (total units/day)	70.8 ± 29.3	72.5 ± 25.5	68.5 ± 36.2
Insulin (mE/L)	27.4 ± 12.5	43.4 ± 25.2	19.4 ± 7.9

Apo, apolipoprotein.*P<0.05 vs dapagliflozin.

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Table 2 Changes in fasting measurements before and after treatment. Data as mean ± s.d. or median (interquartile range). P-model by ANCOVA with post-treatment levels and diabetes duration as dependent variables and pre-treatment levels as covariate and treatment group as fixed effect.

	Dapagliflozin (*n* = 8)		**Placebo** (*n* = 6)		**Between group difference**	
	Baseline	Week 12	Baseline	Week 12	% median change from baseline	p-model
HbA1c (mmol/mol)	66.6 ± 10.7	64.6 ± 13.1	65.2 ± 14.9	61.0 ± 15.0	+1.7	0.77
Glucose (mmol/L)	9.6 ± 3.0	8.7 ± 2.7	9.8 ± 3.4	9.9 ± 2.3	−9.8	0.39
Insulin (mE/L)	43.3 ± 25.2	46.8 ± 23.6	19.4 ± 7.9	21.8 ± 6.0	+9.8	0.12
Insulin (units/day)	72.5 ± 25.5	65.0 ± 28.8*	68.5 ± 36.2	70.0 ± 35.4	−14.8	0.03
Glucagon (pg/mL)	83.1 ± 13.8	95.5 ± 12.2*	110.8 ± 35.8	107.3 ± 18.8	+7.8	0.41
Systolic blood pressure (mmHg)	134.0 ± 9.9	130.5 ± 6.4*	148.2 ± 20.6	146.7 ± 7.1	−1.9	0.04
Weight (kg)	95.8 ± 9.3	94.3 ± 9.5	96.0 ± 12.4	97.7 ± 13.8	−1.2	0.79
Total cholesterol (mmol/L)	3.9 ± 0.7	3.7 ± 0.6	3.6 ± 0.7	3.9 ± 0.7	−14.7	0.45
HDL-cholesterol (mmol/L)	1.0 ± 0.2	1.0 ± 0.3	1.1 ± 0.2	1.0 ± 0.2	+2.9	0.15
LDL-cholesterol (mmol/L)	2.5 ± 0.6	2.0 ± 0.4*	1.9 ± 0.6	1.9 ± 0.2	−25.4	0.48
Triglycerides (mmol/L)	1.13 (0.84–1.42)	1.34 (0.90–1.78)	1.33 (0.75–1.91)	1.45 (0.04–2.86)	+9.4	0.06
Apolipoprotein AI (g/L)	1.27 ± 0.18	1.24 ± 0.18	1.30 ± 0.23	1.23 ± 0.17	+2.1	0.64
Apolipoprotein B (g/L)	0.81 ± 0.15	0.79 ± 0.17	0.72 ± 0.17	0.74 ± 0.19	+4.9	0.94
Apolipoprotein B48 (mg/L)	11.8 (8.6–14.9)	7.4 (3.8–11.0)	9.3 (3.7–14.9)	9.1 (0.6–17.6)	−39.8	0.01
Free fatty acids (mmol/L)	0.51 ± 0.17	0.54 ± 0.15	0.52 ± 0.15	0.55 ± 0.28	+3.9	0.29
Beta-hydroxybutyrate (mmol/L)	0.10 (0.09–0.11)	0.10 (0.08–0.12)	0.10 (0.08–0.13)	0.10 (0.05–0.15)	+1.2	0.67

*$P < 0.05$ vs baseline
HbA1c, hemoglobin A1c; MFI, mean fluorescence index.

p-model = 0.06), and plasma apoB (+4.9%; p-model = 0.94) compared to placebo. Treatment with dapagliflozin did not result in significant changes in vascular function or inflammatory parameters (see Supplementary Table 1, see section on supplementary materials given at the end of this article).

Postprandial effects

Treatment with dapagliflozin resulted in a significant increase in the postprandial response of glucagon (median change +13.7%; p-model = 0.03) and bHBA (+43.8%; p-model = 0.02) (Fig. 1 and Table 3). No significant postprandial effects of dapagliflozin on total plasma triglycerides, apo B48, glucose, insulin, vascular response (Supplementary Fig. 1 and Supplementary Table 2) and inflammatory parameters (Supplementary Fig. 3 and Supplementary Table 2) were observed.

Discussion

In this proof-of-concept study, we investigated the effects of dapagliflozin on postprandial inflammatory and cardiometabolic parameters in patients with T2DM in order to get more insight into the dynamic changes during the postprandial period and to identify possible cardioprotective mechanisms of dapagliflozin in the postprandial situation. Surprisingly, 12 weeks of treatment with dapagliflozin resulted in lower fasting levels of apoB48 reflecting lower levels of postabsorptive chylomicron remnants, without affecting the acute postprandial response. Furthermore, a significant increase in the postprandial response of glucagon and bHBA in our study population was found by dapagliflozin compared to placebo.

The observed decrease in postabsorptive apoB48-containing remnants in T2DM has not been reported before after treatment with an SGLT2i. This could suggest that dapagliflozin does not influence the production or postprandial catabolism of intestinally derived lipoproteins, but that it may enhance the clearance of these particles. An explanation is not readily available at this moment, since SGLT2i are not known to upregulate any of the receptors involved in remnant clearance. In theory, improved insulin sensitivity by dapagliflozin may have improved remnant clearance (18, 19). Our data do not show an effect during the early postprandial period, since apoB48 levels decreased more rapidly in the late postprandial phase by dapagliflozin. This may have resulted in lower fasting levels. The logical explanation would be increased (receptor mediated) clearance in the late postprandial and postabsorptive phases. One study in mice treated with canagliflozin showed an increased activity of

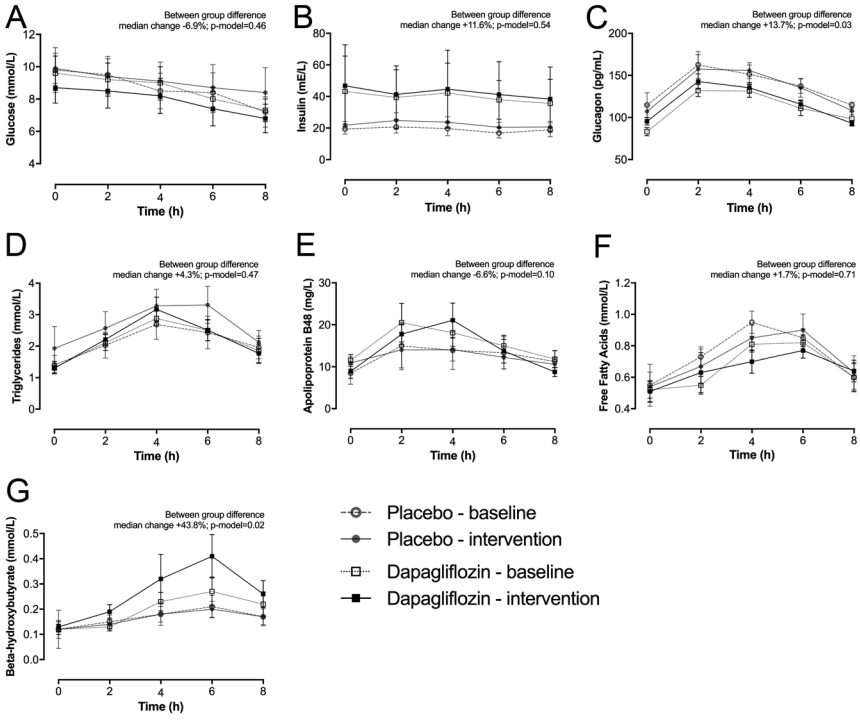

Figure 1

Postprandial response of glucose, insulin, glucagon, triglycerides, apolipoprotein B48, free fatty acids and beta-hydroxybutyrate concentrations after an oral fat load test. Mean (S.E.M.) of glucose (A), insulin (B), glucagon (C), triglycerides (D), ApoB48 (E), free fatty acids (F) and beta-hydroxybutyrate (G) at baseline (open square) and after treatment (closed square) with dapagliflozin and at baseline (open circle) and after treatment (closed circle) with placebo during an oral fat load test. P-model by ANCOVA with post-treatment levels as dependent variable and pre-treatment levels and diabetes duration as covariates and treatment group as a fixed effect.

lipoprotein lipase (LPL) (20). That study showed that when SGLT2 was inhibited via antisense oligonucleotides, a reduced RNA-expression of LDLR was observed, although there was no effect on LDLR activity by SGLT2-inhibition via canagliflozin. Unfortunately, to our knowledge, no data on the activity of LPL in humans by SGLT2-inhibition is available. In our study, the postprandial TG levels did not change making a significant effect on LPL less likely. Alternatively, it has been shown that lipoprotein clearance can be stimulated by SGLT2i by higher heparan sulfate

Table 3 Postprandial (area under the curve$_{0\text{-}8h}$) measurements before and after treatment. Data as mean ± S.D. or median (interquartile range). P-model by ANCOVA with post-treatment levels and diabetes duration as dependent variables and pre-treatment levels as covariate and treatment group as fixed effect. There were no significant differences between groups for baseline and week 12 levels.

	Dapagliflozin (n = 8)		**Placebo** (n = 6)		**Between group difference**	
	Baseline	Week 12	Baseline	Week 12	% median change from baseline	p-model
Glucose (mmol/L × 8 h)	71.3 ± 21.2	65.9 ± 19.6	72.7 ± 23.0	68.9 ± 21.5	−6.9	0.46
Insulin (mE/L × 8 h)	185.6 (120.0–251.2)	193.0 (96.1–289.9)	348.8 (205.4–492.2)	353.8 (226.1–481.5)	+11.6	0.54
Glucagon (pg/mL × 8 h)	465.4 ± 25.4	504.7 ± 18.6*	568.2 ± 26.9	540.2 ± 18.6	+13.7	0.03
Total cholesterol (mmol/L × 8 h)	32.2 ± 5.9	30.4 ± 5.4	30.0 ± 5.9	31.7 ± 5.3	−2.0	0.83
HDL-cholesterol (mmol/L × 8 h)	8.1 ± 1.8	8.3 ± 1.9	8.6 ± 1.7	8.4 ± 1.6	+6.7	0.44
Triglycerides (mmol/L × 8 h)	17.0 ± 5.9	18.9 ± 5.0	17.9 ± 7.1	19.1 ± 9.5	+4.3	0.47
Apolipoprotein B48 (mg/L × 8 h)	116.9 (86.2–147.6)	84.0 (52.0–116.0)	125.7 (47.4–204.0)	114.4 (83.0–197.4)	−6.6	0.10
Free fatty acids (mmol/L × 8 h)	5.3 ± 0.8	6.3 ± 1.3	5.5 ± 1.3	6.3 ± 1.6	+1.7	0.71
Beta-hydroxybutyrate (mmol/L × 8 h)	1.57 (0.60–2.54)	2.34 (1.30–3.38)*	1.15 (0.69–1.61)	0.91 (0.57–1.57)	+43.8	0.02

MFI, mean fluorescence index.*P < 0.05 vs baseline

proteoglycans HSPG-dependent pathways. In animal models, these pathways could be activated by SGLT2i resulting in reduced atherosclerosis (21). In that same study, a role of the LDLR, the low-density lipoprotein receptor-related protein 1, and the scavenger receptor class B were excluded. Our data in patients with T2DM support these observations. In T2DM, insulin resistance increases the postprandial production of chylomicrons and reduces chylomicron remnant clearance, leading to the accumulation of chylomicron remnants in the circulation (22, 23, 24). It has been established that postprandial hyperlipidemia and remnants are associated with an increased risk for cardiovascular disease (25, 26) and this has also been demonstrated in patients with T2DM (27). Altogether, lower apoB48 levels may reflect a novel anti-atherosclerotic effect of dapagliflozin which in part may explain the observed beneficial cardiovascular effects seen in the different trials. Additional studies with tracers could provide more insight is these mechanisms.

Our study also shows that there is no effect of SGLT2i on postprandial inflammatory markers. This may not be surprising since postprandial inflammation is closely linked to the postprandial chylomicron (remnant) response (28), and postprandial apoB48 levels were unaffected. Therefore, decreased postprandial inflammation does not seem to be one of the mechanisms involved in cardiovascular protection by dapagliflozin.

There is limited evidence that treatment with SGLT2i results in an increase in ketone body formation (mainly bHBA) (9, 29, 30). In our study, we did not find a significant change in fasting levels of bHBA after treatment with dapagliflozin. Nevertheless, we did observe a significant higher postprandial response of bHBA. It might be that increased hepatic FFA catabolism, reflected by higher ketone bodies, becomes more evident in the postprandial situation (31). This increase in postprandial bHBA levels could be explained by either an increased hepatic influx of chylomicron-derived or adipose tissue-derived FFA and/or upregulation of the hepatic beta-oxidation machinery. In theory, lower insulin use by the participants may have led to less inhibition of adipose tissue lipolysis and the higher postprandial glucagon levels may have induced increased adipose tissue lipolysis (32). The consequence may have been an increased postprandial fatty acid flux from the adipose tissue to the liver. Therefore, we cannot clearly identify in which tissue the most prominent effects of dapagliflozin were found.

It has been suggested that increased ketone bodies may improve cardiac function and that this may in part explain the rapid improvement in cardiovascular outcomes in the SGLT2i trials. Especially, the beneficial effects on heart failure hospitalization in these trials have been linked to higher ketone bodies (29, 30). Our study suggests that this increased ketogenesis may especially occur in the postprandial period.

Previous studies have explored the effect of SGLT2i on glucagon levels and found higher fasting or postprandial glucagon levels (9, 33). These results were confirmed in the present study. Whether the increased glucagon response after dapagliflozin treatment is linked to the decreased apoB48 levels remains to be elucidated. It has been shown that hyperglucagonemia in healthy volunteers has no effect on intestinal lipoprotein metabolism (34), but whether this is also the case in T2DM is unknown.

One major limitation of our study is the small number of subjects included. We aimed to include 20 patients in total; unfortunately, due to problems with recruitment and deadlines we had to stop the study after 14 included patients. However, the detailed metabolic information obtained is robust and increases our knowledge on the effects of SGLT2i on lipid metabolism. Another limitation of our study is the fact that we did not directly quantitate both fatty acid oxidation, adipose tissue lipolysis and lipoprotein turnover. As such, we did not demonstrate whether the dapagliflozin-induced decrease in fasting apoB48 levels is a consequence of lower intestinal production or higher catabolism, although the postprandial curves suggest a lack of effect on the acute postprandial production. Furthermore, our patients were treated with intensive insulin therapy representing not only insulin resistance but also some degree of beta-cell dysfunction. Finally, despite randomization, a significant difference in diabetes duration between treatment groups was observed. In order to address this issue, diabetes duration was added as a covariate in the main analyses.

In conclusion, we showed that 12 weeks of treatment with dapagliflozin decreased the postabsorptive levels of atherogenic chylomicron remnants and increased the postprandial response of bHBA compared to placebo against a background of higher glucagon levels and lower insulin doses. Lower levels of postabsorptive chylomicron remnants and increased postprandial levels of bHBA may explain in part the cardiovascular protection seen in large clinical trials with this type of drugs. However, this needs to be confirmed in larger trials including these parameters.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EJE-21-1270.

Declaration of interest
Manuel Castro Cabezas served as a member of different advisory boards and received fees for educational lectures from Boehringer Ingelheim, Astra Zeneca and Mundipharma. The other authors report no conflicts of interest.

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Author contribution statement
B B, N M C P, S F A and L C v V v d Z performed the investigation. B B, N M C P, G J M v d G, E B, E M v d Z and M C C developed the methodology. J H, M T M, P C N R provided resources. E B, W W d H, M T M, P C N R reviewed and edited the manuscript. B B formally analyzed and curated the data, wrote the first draft of the manuscript, reviewed and edited the manuscript and provided visualizations. M C C is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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