The effect of dapagliflozin on apolipoprotein B and glucose fluxes in patients with type 2 diabetes and well-controlled plasma LDL cholesterol

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Abstract
Aim: To dissect the effects of the sodium-glucose linked transporter 2 inhibitor dapagliflozin on lipid metabolism and assess whether these effects could potentially offset cardiovascular benefit with this drug-class.

Materials and Methods: We assessed the effect of dapagliflozin on lipid metabolism in 11 adults with uncomplicated type 2 diabetes. After 4 weeks of statin wash-out and 4 weeks of rosuvastatin 10 mg treatment, participants were treated with dapagliflozin 10 mg once-daily for 5 weeks. Before and after dapagliflozin, plasma lipids were measured and very low-density lipoprotein (VLDL)-1 and VLDL-2 apolipoprotein (Apo)B fluxes were assessed using (5,5,5-2H3)-leucine tracer infusion. In addition, hepatic and peripheral insulin sensitivity as well as insulin-mediated inhibition of peripheral lipolysis were measured during a two-step hyperinsulinemic-euglycaemic clamp using (6,6-2H2)-glucose and (1,1,2,3,3-2H5)-glycerol tracers.

Results: Rosuvastatin decreased all plasma lipids significantly: total cholesterol from 4.5 (3.2–6.2) to 3.1 (2.5–3.8) mmol/L, LDL cholesterol from 2.6 (1.7–3.4) to 1.5 (1.1–2.2) mmol/L, HDL cholesterol from 1.34 (0.80–2.02) to 1.19 (0.74–1.89) mmol/L and triglycerides from 0.92 (0.31–3.91) to 0.79 (0.32–2.10) mmol/L. The addition of dapagliflozin to rosuvastatin did not raise either LDL cholesterol or total cholesterol, and only increased HDL cholesterol by 0.08 (–0.03–0.13) mmol/L (P = 0.03). In line with this, dapagliflozin did not affect VLDL-1 or VLDL-2 ApoB fluxes. Fasting endogenous glucose production tended to increase by 0.9 (–3.4–3.1) μmol kg⁻¹ min⁻¹.
INTRODUCTION

Adults with type 2 diabetes have an increased risk of developing cardiovascular disease (CVD), leading to increased mortality in this population. This increased risk can, at least in part, be attributed to the atherogenic lipid profile in this population. Most people with type 2 diabetes are therefore treated with statins in an attempt to normalize these pathophysiologic changes. Reducing hyperglycaemia by traditional glucose-lowering agents, such as metformin, sulfonylurea and insulin, does not appear to reduce this CVD burden, particularly in patients with long-standing disease and some glucose-lowering drugs have even been linked to increased CVD. Although glucagon-like peptide-1 receptor agonists are today also known to improve cardiovascular (CV) outcome, the introduction of the sodium-glucose co-transporter-2 (SGLT-2) inhibitors was met with great enthusiasm. These agents were designed to block the reuptake of glucose in the renal proximal tubule, thereby inducing glucosuria and reducing hyperglycaemia. Because of concomitant inhibition of sodium reabsorption, SGLT-2 inhibitors induce transient natriuresis, which, together with continuous osmotic diuresis, is associated with plasma volume contraction. This reduction in plasma volume, as well as salutary effects on blood pressure, body weight and uric acid, probably drives the CV benefit of SGLT-2 inhibitors in adults with type 2 diabetes. Other possible explanations include a switch from carbohydrates towards free fatty acid (FFA) and ketone bodies as myocardial substrates towards free fatty acid (FFA) and ketone bodies as myocardial substrates, an inhibition of hepatic LDL receptor (LDLr) expression but also decreased intestinal cholesterol uptake, which may lead to increased cholesterol synthesis and secretion of very-low density lipoprotein (VLDL). Together, this will result in increased circulating LDLc levels. Currently, there are no data available in humans to explain the SGLT-2 inhibitor-induced increment in plasma LDLc concentrations. Thus, we investigated the mechanisms by which the SGLT-2 inhibitor dapagliflozin affects plasma LDLc concentrations in adults with uncomplicated type 2 diabetes on standardized statin background therapy using VLDL-1 and VLDL-2 apolipoprotein (apo)B fluxes employing stable isotopes. We also assessed the effect of dapagliflozin on hepatic and peripheral glucose fluxes as well as on energy metabolism and peripheral lipolysis.

MATERIALS AND METHODS

Study participants

Male and postmenopausal female adults with type 2 diabetes were recruited by newspaper advertisements. Eligible individuals were on stable metformin treatment for at least 3 months, were aged 18–80 years and had an HbA1c of 48–69 mmol/mol or 6.5%–8.5%. Individuals with a history of CVD, smoking, the use of any glucose-lowering agent other than metformin, alcohol abuse (>3 units/day), aspartate aminotransferase or alanine aminotransferase elevation (>2.5-fold the upper limit) or an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73m² were excluded. Body weight and food intake were stable 3 months prior to inclusion. Written informed consent was obtained from all persons before any trial-related activities. The trial was registered at ClinicalTrials.gov (NCT03074630) and was approved by the local Institutional Review Board of the Amsterdam University Medical (AMC) Centres, location AMC, the Netherlands. This study was conducted in accordance with the Declaration of Helsinki as revised in 2008.

Conclusions: Dapagliflozin has no effect on plasma LDL-cholesterol levels or VLDL-apoB fluxes in the context of optimal lipid-lowering treatment, which will thus not limit cardiovascular benefit when lipids are adequately controlled.

KEYWORDS

apolipoprotein B, dapagliflozin, glucose, insulin sensitivity, LDL cholesterol, lipolysis, sodium-glucose co-transporter-2 inhibitor, type 2 diabetes

An elegant study in streptozotocin-treated mice indeed showed that SGLT-2 inhibition increases plasma levels of LDLc because of impaired LDLr-mediated hepatic clearance of LDL as well as greater lipoprotein lipase (LPL)-mediated lipolysis of TG in TG-rich lipoproteins. A similar potential mechanism comes from a study in diet-induced dyslipidaemic hamsters, where the SGLT-2 inhibitor empagliflozin caused an inhibition of hepatic LDL receptor (LDLr) expression but also decreased intestinal cholesterol uptake, which may lead to increased cholesterol synthesis and secretion of very-low density lipoprotein (VLDL).
2.2 Study design

After study inclusion at screening visit, lipid-lowering therapy was discontinued for 4 weeks. After this wash-out period, participants started with 4 weeks of rosuvastatin 10 mg once-daily to standardize lipid-lowering therapy. Then the actual intervention phase commenced, during which patients were treated with 10 mg dapagliflozin once-daily for 5 weeks while rosuvastatin was continued. Before and at week 5 of dapagliflozin treatment, VLDL-1 and VLDL-2 apoB fluxes were assessed by (5,5,5-$^{2}$H$_{3}$)-leucine. Hepatic and peripheral insulin sensitivity using (6,6-$^{2}$H$_{2}$) glucose and peripheral lipolysis using (1,1,2,3,3-$^{2}$H$_{5}$)-glycerol were measured during a two-step hyperinsulinemic-euglycaemic clamp, as described below (Figure 1). Insulin sensitivity was also divided by insulin concentrations during the clamp to correct for potential differences between test days/participants.

2.3 ApoB VLDL-1 and VLDL-2 fluxes

After an overnight fast, blood samples were drawn before 7 mg/kg body weight of (5,5,5-$^{2}$H$_{3}$)-leucine (99% enriched; Cambridge Isotope Laboratories, Andover, MA) was infused via a venous catheter. At 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360, 480, 600 and 1340 minutes after infusion, blood samples were drawn for the determination of (5,5,5-$^{2}$H$_{3}$)-leucine enrichment in VLDL-1 and VLDL-2 fractions. VLDL-1 and VLDL-2 fractions were isolated from plasma by a three-step gradient ultracentrifugation using a SW41 rotor (Beckman). Briefly, the density of 4 mL of plasma was adjusted to 1.1502 g/mL with NaCl. Then 0.5 mL NaBr/NaCl (d = 1.182 g/mL) and 4 mL plasma (d = 1.1502 g/mL) were transferred into an ultraclear Beckman SW41 tube. Next, the gradient was formed by layering the following salt solutions on top of the plasma: (1) 2 mL, 1.079 g/mL; (2) 2 mL, 1.0722 g/mL; (3) 2 mL, d = 1.0641, g/mL; (4) 2 mL, d = 1.0588 g/mL. The different fractions were then isolated by

FIGURE 1 Overview of (A) study protocol and individual study days, (B) two-step hyperinsulinemic euglycaemic clamp day, and (C) overview of very low-density lipoprotein (VLDL)-HDL kinetics. REE, resting energy expenditure
sequential centrifugation in a Beckman ultracentrifuge. The following centrifugation times were applied: for chylomicrons: 30 minutes, 39,000 rpm; for VLDL-1: 51 minutes, 39,000 rpm; and for VLDL-2: 16.22 hours, 18,000 rpm. Each time, the upper 1 mL was aspirated and replaced by the appropriate density fraction. Fractions were frozen at 
−80°C for further analysis. For leucine enrichment analysis, VLDL-1 and VLDL-2 samples were precipitated with isopropanol, delipidated with ethanol-diethyl ether, then dried and hydrolysed with 6 M HCl at 110°C for 24 hours. The samples were then prepared for analysis of leucine enrichment, as described using norleucine as an internal standard. Enrichments were determined by Gas Chromatography–Mass Spectrometry (GC–MS) GC-MSD5975c (Agilent Technologies) equipped with a very-low-flow-loss column 17 MS column operated in Selective Ion Monitoring mode. To calculate isotope enrichments, the average value of the m/z 161:158 ratio was determined using a calibration curve with known quantities of labelled and unlabelled leucine. The resulting m/z 161:158 was expressed as a molar percentage ratio (MPE).

### 2.4 | Glucose fluxes and peripheral lipolysis

After another overnight fast, catheters were inserted into a distal vein of both arms. One catheter was used for the infusion of the (6,6-2H2) glucose and (1,1,2,3,3-2H5)-glycerol tracer (both >99% enriched; Cambridge Isotope Laboratories). The other catheter was used for arterialized blood sampling using a heated hand box. Blood samples were drawn for determination of background enrichments 2 hours before the clamp started. Then a continuous infusion of both (6,6-2H2) glucose (prime, 11 μmol/kg; continuous, 0.11 μmol·kg⁻¹·min⁻¹) and (1,1,2,3,3-2H5)-glycerol (prime, 1.6 μmol/kg; continuous, 0.11 μmol·kg⁻¹·min⁻¹) was started and continued until the end of the experiment. The infusion of insulin (step 1 at 20 mU·m⁻²·min⁻¹ actrapid 100 IU/mL; Novo Nordisk Farma, Alphen aan den Rijn, the Netherlands) was preceded by triple blood sampling, at 5 minute intervals, to assess the enrichment of glucose and glycerol and to measure glucoregulatory hormones in the basal state. During insulin infusion, plasma glucose was monitored and 20% glucose, supplemented with (6,6-2H2) glucose to maintain tracer equilibrium, was infused at variable rates to maintain blood glucose at 5 mmol/L. After 2 hours, five blood samples were drawn at 5 minute intervals to determine glucose and glycerol enrichments, glucoregulatory hormones and free fatty acids (FFA). After 2.35 hours, insulin infusion continued at 60 mU·m⁻²·min⁻¹ (step 2) for 2 hours, after which blood sampling was repeated. Resting energy expenditure (REE) was assessed by indirect calorimetry during the start of the second step of the clamp for a duration of 20 minutes. Oxygen consumption and CO2 production was measured using a ventilated hood system (Sensormedics, Anaheim, CA). REE was calculated as previously described.

### 2.5 | Biochemical analyses

Plasma glucose concentration was determined with the glucose oxidation method using a Biosen C-Line plus glucose analyser (EFK Diagnostics, Fullerton, CA). Insulin and cortisol were determined by immunoassay on an Immulite 2000 system (Diagnostic Products, Los Angeles, CA) with an intra-assay variation of 4%-5% and 3%-6%, respectively. Glucagon was determined by radioimmunoassay (Linco Research, St Charles, MO) with an intra-assay variation of 4%-8%. Plasma FFA was analysed by an enzymatic colorimetric assay (NEFA-C kit, Wako Chemicals, Neuss, Germany). Plasma total cholesterol, LDL cholesterol, HDL cholesterol and TG were analysed using commercial assays (Roche). Plasma ApoB and Apol were analysed by immunoturbidimetric assay (Wako Chemicals) with a Selecta auto-analyser (Sopachem). Plasma enrichments of (6,6-2H2) glucose and (1,1,2,3,3-2H5)-glycerol were determined by GC-MS.

### 2.6 | Calculations

Endogenous glucose production (EGP) and the peripheral uptake of glucose (rate of disappearance [Rd]) were calculated using modified versions of Steele equations for the non-steady state and are expressed as μmol·kg⁻¹·min⁻¹. Hepatic insulin sensitivity was calculated as the percentage suppression of basal EGP during the first step of hyperinsulinemia. Urinary glucose excretion during the clamps was subtracted from total glucose disposal before calculating glucose fluxes. Lipolysis was computed using formulae for steady-state kinetics adapted for stable isotopes and is expressed as μmol·kg⁻¹·min⁻¹.

### 2.7 | Statistical analyses

Data were analysed with Wilcoxon signed rank test. Non-parametric analyses were chosen because of the small number of participants. Regarding sample size calculations, based on previous studies, a LDL-apoB synthesis rate of 1.8 ± 0.35 g/day was assumed. Furthermore, it was expected that SGLT-2 inhibition would increase this by 0.3 ± 0.35 g/day. To achieve 80% power with an alpha set at 0.05 (single-sided), 11 subjects needed to be included. Taking into account a 10% drop-out rate, we aimed to include 12 participants. All statistical analyses were performed using SPSS software version 25. A P-value of <0.05 was considered statistically significant. Data are represented as median (min.-max.) unless otherwise specified.

### 3 | RESULTS

#### 3.1 | Subject characteristics

Nine male and three postmenopausal female participants were included. One female participant was excluded during the study because of missing data, as we were unable to place a venous catheter during the second hyperinsulenic clamp, thus we present the analysis for the 11 evaluable participants. Baseline characteristics are depicted in Table 1.
3.2 | Effect of rosuvastatin on lipid variables

Rosuvastatin decreased all cholesterol variables significantly (Table 2): ApoB by 44 (−78 to −12) mg/dl (P = 0.02), ApoAI by 10 (−33 to 4) mg/dl (P = 0.03), total cholesterol by 1.3 (−2.8 to −0.2) mmol/L (P < 0.01), LDLc by 1.1 (−2.1 to −0.2) mmol/L (P < 0.01), HDLc by 0.09 (−0.32 to 0.07) mmol/L (P = 0.02) and TG by 0.21 (−1.81 to 0.32) mmol/L (P = 0.02).

3.3 | Effect of dapagliflozin on plasma cholesterol and lipid metabolism

The addition of dapagliflozin to rosuvastatin did not affect plasma total cholesterol, LDLc or TG levels, but it did increase plasma HDLc by 0.08 (−0.03 to 0.13) mmol/L (P = 0.03; Table 2). In line with the observed effects on plasma lipids, the addition of dapagliflozin to rosuvastatin did not affect plasma ApoB or ApoAI (Table 2). Regarding the leucine-apoB fluxes, the enrichment in VLDL-1 rapidly increased because of the greater influx of labelled leucine in newly synthesized apoB compared with the outflux. After the peak there is a rapid decay of the curve. At the same time the VLDL2 enrichment increases, because of material derived from VLDL-1 or from direct synthesis through the liver. The incorporation of labelled leucine into apoB in VLDL-1 and VLDL-2 was not altered by dapagliflozin (Figure 2B).

3.4 | Effect of dapagliflozin on glucose metabolism and lipolysis

Dapagliflozin slightly reduced body weight from 97 (80–134) to 96 (78–134) kg (P = 0.02). Dapagliflozin lowered fasting plasma glucose by 0.6 (−1.9 to 0.4) mmol/L (P = 0.06). HbA1c was reduced from 49 (47–61) to 47 (41–52) mmol/mol or from 6.6% (6.5%–7.7%) to 6.5% (5.9%–6.9%) (P < 0.01). Fasting insulin was reduced by 7 (−43 to 22) pmol/L (P = 0.04), while fasting glucagon was increased by 8 (−7 to 20) ng/L (P = 0.03). Dapagliflozin increased fasting EGP by 0.9 (−3.1 to 3.3) μmol kg⁻¹ min⁻¹ (P = 0.06; Figure 2C). Hepatic insulin sensitivity (expressed as percentage of EGP suppression; Figure 2D) and peripheral insulin sensitivity (Rd, Figure 2E) were not affected by dapagliflozin. Insulin levels were 203 (138–370) and 233 (127–327) pmol/L, respectively, before and after dapagliflozin treatment during the first step of the clamp. When leaving out one outlier from the EGP analyses, the increase in EGP (P = 0.017) and decrease in EGP suppression (P = 0.037) became significant. During the second step, the levels were 603 (350–1150) and 545 (300–1090) pmol/L, respectively. Correcting glucose fluxes for insulin levels did not alter the effect on peripheral insulin sensitivity (Table 3). Glucagon levels were 57 (43–96) and 60 (43–86) ng/L, respectively, before and after dapagliflozin treatment during the first step of the clamp and 52 (39–75) and 54 (38–81) ng/L during the second step. Lastly, dapagliflozin increased fasting plasma FFA concentrations by 0.20 (0.04 to 0.78) mmol/L (P = 0.03), but had no effect on peripheral lipolysis during the first clamp phase (Ra, Figure 2F) or REE during the second clamp phase (Table 3).

### TABLE 1 Baseline characteristics

| Characteristic                  | Study participants (n = 11) |
|--------------------------------|-----------------------------|
| Age, years                     | 64 (50–76)                  |
| Male sex, n (%)                | 9 (81)                      |
| Diabetes duration, years       | 11 (2–13)                   |
| Body weight, kg                | 96 (82–135)                 |
| BMI, kg/m²                     | 30.5 (25.8–38.3)            |
| Fasting plasma glucose, mmol/L | 7.5 (6.7–12.4)              |
| HbA1c, mmol/mol                | 49 (44–59)                  |
| HbA1c, %                       | 6.6 (6.2–7.5)               |
| Systolic blood pressure, mmHg  | 134 (109–175)               |
| Diastolic blood pressure, mmHg | 83 (76–99)                  |
| eGFR (CKD-EPI), ml/min/1.73m²  | 81 (63–101)                 |
| RAS blockade, n (%)            | 6 (55)                      |

Abbreviations: BMI, body mass index; CKD-EPI, Chronic Kidney Disease-Epidemiology collaboration; eGFR, estimated glomerular filtration rate; RAS, renin-angiotensin system.

Data are represented as median (min.-max.) or n (%).

### TABLE 2 Plasma cholesterol levels at baseline, with 4 weeks of rosuvastatin and after the addition of dapagliflozin for 5 weeks

|                    | Before rosuvastatin (week 0) | With rosuvastatin (week 4) | P-value (vs. week 0) | With dapagliflozin (week 9) | P-value (vs. week 4) |
|--------------------|------------------------------|----------------------------|----------------------|----------------------------|----------------------|
| ApoB, mg/dl        | 94 (67–118)                  | 53 (40–68)                 | 0.02                 | 52 (39–64)                 | 0.94                 |
| ApoAI, mg/dl       | 165 (111–192)                | 147 (104–182)              | 0.03                 | 151 (106–193)              | 0.18                 |
| Total cholesterol, mmol/L | 4.5 (3.2–6.2)            | 3.1 (2.5–3.8)              | <0.01                | 3.0 (2.7–3.7)              | 0.86                 |
| LDLc, mmol/L       | 2.6 (1.7–3.4)                | 1.5 (1.1–2.2)              | <0.01                | 1.4 (0.9–1.9)              | 0.23                 |
| HDLc, mmol/L       | 1.3 (0.8–2.0)                | 1.2 (0.7–1.9)              | 0.02                 | 1.2 (0.8–2.0)              | 0.03                 |
| Triglycerides, mmol/L | 0.9 (0.3–3.9)               | 0.8 (0.3–2.1)              | 0.02                 | 0.9 (0.4–1.9)              | 0.63                 |

Abbreviations: ApoAI, apolipoprotein AI; ApoB, apolipoprotein B; HDLc, HDL-cholesterol; LDLc, LDL-cholesterol.

Data are presented as median (min.-max.). The timepoints were compared using Wilcoxon signed rank tests. Significant differences are indicated in bold.
3.5 | Adverse events

Dapagliflozin was well tolerated in all 12 patients. There were no hypoglycaemic events; one participant experienced complaints of vaginal candidiasis, which was successfully treated. There were no other side effects related with dapagliflozin during the short treatment period.

4 | DISCUSSION

This study shows for the first time that dapagliflozin as add-on to metformin therapy does not induce unfavourable changes in plasma lipid concentrations or apoB kinetics in adults with uncomplicated type 2 diabetes and adequately controlled plasma LDLc. Most notably, we observed no increment in plasma LDLc concentrations with dapagliflozin treatment, while we did see a slight increase in fasting plasma FFA. Finally, we observed no changes in insulin sensitivity, while fasting EGP was increased, as reported previously.27,28

This study was carried out to investigate the underlying mechanisms by which SGLT-2 inhibitors lead to increased plasma LDLc levels in adults with type 2 diabetes. This ≈ 0.2 mmol/L rise in LDLc, as observed in phase III trials, despite a few kg of weight loss,16 was initially worrisome and somewhat surprising. The results from CV safety trials—in which empagliflozin,29 canagliflozin30 and dapagliflozin31 showed CV benefit over placebo—were reassuring. Overall, these SGLT-2 inhibitors reduced major adverse CV events, composed of non-fatal myocardial infarction or stroke and CV death, by 11% (HR 0.89 [95% CI 0.83–0.96], P = 0.001), and the risk of CV death or hospitalization for heart failure by 23% (0.77 [0.71–0.84], P<0.0001).15 This benefit occurred despite minor LDLc increments with empagliflozin29 and canagliflozin.30 These data are not available for the DECLARE-TIMI trial, but LDLc increments were also observed with dapagliflozin in phase III trials.16 Although the consequences of this rise in LDLc are apparently limited, it could potentially still counteract the CV benefit of these agents. To maximize the gain of this drug class, it is important to understand the underlying mechanisms. Rodent studies showed that SGLT-2 inhibition impaired LDL-mediated hepatic clearance of LDLc, while LPL-mediated lipolysis of TG-rich lipoproteins was increased.19 In addition, studies in hamsters revealed a switch in energy metabolism upon SGLT-2 inhibitor
treatment from carbohydrate to lipid utilization, a decrease in intestinal cholesterol absorption, an increase in hepatic 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase and a decrease in hepatic LDLr expression, together leading to a moderate increase in circulating plasma LDLc levels.20 Because lipid metabolism is influenced by glucose homeostasis and lipolysis, we directly measured lipid fluxes, glucose metabolism and insulin-mediated inhibition of peripheral lipolysis in the same protocol in order to provide an integrative approach.

In contrast to previously indicated data, dapagliflozin did not cause any additional changes in LDLc in our study. A possible explanation for this is that after treating our patients with a robust dose of rosuvastatin only (thus before the initiation of dapagliflozin), plasma LDLc was lowered to 1.5 mmol/L. In the EMPA-REG OUTCOME trial29 and CANVAS programme,30 baseline LDLc concentrations were significantly higher (2.2 and 2.3 mmol/L, respectively) and above recommended targets, despite the fact that 75%–80% of participants were on statin therapy. This difference might be explained by (1) low compliance in the large trials versus our comparatively small trial with strict follow-up, (2) the use of less potent statins, or (3) submaximal dosing of lipid-lowering therapy. Dosages and the type of statin used were not reported in these large trials. A recent update of the American Diabetes Association guidelines on CV risk management advocated that LDLc should be lowered to less than 1.8 mmol/L, at least for people with type 2 diabetes and established CV disease.1 Our results indicate that adhering to the guidelines might prevent the rise in LDLc and enhance the magnitude of SGLT-2 inhibitor-induced CV benefit. SGLT-2 inhibitors seemingly increase fasting lipolysis rates. As such, we found a clear increase in plasma FFA concentrations, which is probably explained by the observed reduction in the insulin/glucagon ratio. We also measured lipolysis by D5 glycerol fluxes under hyperinsulinemic conditions (plasma insulin 200 and 600 pmol/L). Under these standardized insulin concentrations, no differences were observed, which indicates that dapagliflozin does not affect adipose tissue insulin sensitivity per se.

With respect to glucose metabolism, we confirm previously reported increases in fasting EGP with dapagliflozin in concordance with a rise in glucagon and a reduction in insulin concentrations.27,28 We did not, however, find SGLT-2 inhibitor-mediated improvements in insulin sensitivity, either when measured as insulin-mediated glucose disposal or suppression of hepatic glucose production. In a trial with 66 people with type 2 diabetes, acute administration of empagliflozin reduced tissue glucose disposal after a mixed meal, attributed to urinary glucose excretion and lower insulin levels, while insulin sensitivity, which was measured as the ratio of mean glucose metabolic clearance rate to mean insulin concentration, was improved. After 4 weeks of treatment, insulin sensitivity, measured as the ratio of mean glucose metabolic clearance rate to mean insulin concentration during meal absorption, was no longer significantly improved, while tissue glucose disposal was still reduced.27 In line with this, 2 weeks of dapagliflozin treatment improved insulin-mediated glucose disposal by 18% during a hyperinsulinemic-euglycaemic clamp in 12 men with type 2 diabetes, which is probably explained byameliorated glucotoxicity.28 The fact that we did not find an improvement in insulin-mediated glucose disposal after 5 weeks of treatment could be explained by differences in glycemic control at baseline: our participants had a median HbA1c of 49 mmol/mol or 6.6%, leaving little room for improvement, versus 68 mmol/mol or 8.4%.28 Therefore, our data at least indicate that 5 weeks of minimal reductions in glucotoxicity are insufficient to produce improvements in insulin.

### Table 3: The effects of dapagliflozin treatment on metabolic measures before and after dapagliflozin treatment

| Metabolic effects of dapagliflozin | Before dapagliflozin (week 4) | After dapagliflozin (week 9) | P-value |
|-----------------------------------|-------------------------------|-------------------------------|---------|
| Fasting plasma glucose (mmol/L)   | 8.2 (6.8–10.0)                | 7.7 (6.0–11.3)                | 0.06    |
| Fasting glucagon (ng/L)           | 66 (44–88)                    | 74 (47–98)                    | 0.03    |
| Fasting insulin (pmol/L)          | 60 (19–170)                   | 41 (17–170)                   | 0.04    |
| Fasting EGP (μmol kg⁻¹ min⁻¹)     | 11.3 (7.6–14.4)               | 11.2 (9.9–17.3)               | 0.06    |
| Hepatic insulin sensitivity during first clamp phase (% EGP suppression) | 52 (37–76) | 46 (33–100) | 0.18 |
| Peripheral insulin sensitivity, glucose disposal during second clamp phase (μmol kg⁻¹ min⁻¹) | 35 (22–78) | 35 (13–56) | 0.72 |
| Glucose disposal corrected for insulin concentration (μmol kg⁻¹ min⁻¹/pmol L⁻¹) | 0.05 (0.03–0.22) | 0.07 (0.02–0.19) | 0.42 |
| Resting energy expenditure, measured at the start of the second clamp phase (kcal/24 h) | 1686 (1370–2331) | 1825 (1328–2331) | 0.79 |
| Fasting plasma FFA, mmol/L        | 0.44 (0.05–0.73)              | 0.74 (0.46–0.87)              | 0.03    |
| Peripheral lipolysis during first clamp phase (glycerol appearance; μmol kg⁻¹ min⁻¹) | 56 (5–83) | 66 (31–88) | 0.11 |

Abbreviations: EGP, endogenous glucose production; FFA, free fatty acids.

Data are presented as median (min.-max.). The timepoints were compared using Wilcoxon signed rank tests. Significant differences indicated in bold.
sensitivity. Of note, Merovci et al were unable to study insulin-mediated EGP suppression as a measure of hepatic insulin sensitivity because of the high insulin dose during the clamp (80 μU m⁻² min⁻¹).²⁸

To our knowledge, this study is the first to explore the effects of dapagliflozin on both glucose and cholesterol fluxes in the same individuals and thus provides insight into the effects of dapagliflozin on intermediary metabolism. Another strength of our study is that it is clinically relevant, as all participants with type 2 diabetes were on standardized lipid-lowering therapy, which led to strict control of plasma lipids in concordance with current guidelines. Our study was, however, limited by the comparatively small number of participants, the absence of a control group and short follow-up time. We, therefore, had limited power to detect changes in secondary outcomes and the data should thus be considered as hypothesis-generating. Post hoc analyses of larger trials are warranted to confirm that the lipidemic response to SGLT2 inhibition is indeed determined by baseline LDLc levels. Furthermore, we only included subjects with uncomplicated type 2 diabetes on metformin monotherapy, which means that our findings cannot be extrapolated to people with complicated or more advanced disease.

In conclusion, we show that dapagliflozin treatment does not alter ApoB or plasma cholesterol levels in people with uncomplicated type 2 diabetes on rosvastatin background therapy. Reassuringly, the potential adverse effect of an SGLT-2 inhibitor-induced rise in LDLc as found in other trials, does not appear to be an issue in this population when lipid-lowering therapy is optimized.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

KB, EvB, MN, GD and DvR designed and set up the trial. KB, EvB, HJ, DvH, HS, MA, MS, AS and GD were involved in sample collection and/or analysis. KB, EvB and GD performed statistical analysis. KB, EvB and DvR wrote the first draft of the paper, all the other authors commented on this draft and the submitted version was approved by all authors. KB is the guarantor of this manuscript. She had full access to the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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