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Clinical Research Article

Emergence of a New Glucoregulatory Mechanism for Glycemic Control With Dapagliflozin/Exenatide Therapy in Type 2 Diabetes

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Abstract

Context: This study addresses the development of a new glucoregulatory mechanism in type 2 diabetes (T2D) patients treated with SGLT-2 inhibitors, which is independent of glucose, insulin and glucagon. The data suggest the presence of a potential trigger factor (s) arising in the kidney that stimulates endogenous glucose production (EGP) during sustained glycosuria.

Objective: To investigate effects of SGLT-2 inhibitor therapy together with GLP-1 receptor agonist on EGP and glucose kinetics in patients with T2D. Our hypothesis was that increased EGP in response to SGLT2i-induced glycosuria persists for a long period and is not abolished by GLP-1 RA stimulation of insulin secretion and glucagon suppression.

Methods: Seventy-five patients received a 5-hour dual-tracer oral glucose tolerance test (OGTT) (intravenous 3-(^{3}H)-glucose oral (1-^{14}C)-glucose): (1) before/after 1 of dapagliflozin (DAPA); exenatide (EXE), or both, DAPA/EXE (acute study), and (2) after 1 and 4 months of therapy with each drug.

Results: In the acute study, during the OGTT plasma glucose (PG) elevation was lower in EXE ($\Delta = 42 \pm 1$ mg/dL) than DAPA ($\Delta = 72 \pm 3$), and lower in DAPA/EXE ($\Delta = 11 \pm 3$) than EXE and DAPA. EGP decrease was lower in DAPA ($\Delta = -0.65 \pm 0.03$ mg/kg/min) than EXE ($\Delta = -0.96 \pm 0.07$); in DAPA/EXE ($\Delta = -0.84 \pm 0.05$) it was lower than EXE, higher than DAPA. At 1 month, similar PG elevations (EXE, $\Delta = 26 \pm 1$ mg/dL; DAPA, $\Delta = 62 \pm 2$, DAPA/EXE, $\Delta = 27 \pm 1$) and EGP decreases (DAPA, $\Delta = -0.60 \pm 0.05$ mg/kg/min; EXE, $\Delta = -0.77 \pm 0.04$; DAPA/EXE, $\Delta = -0.72 \pm 0.03$) were observed. At 4 months, PG elevations (EXE, $\Delta = 55 \pm 2$ mg/dL; DAPA, $\Delta = 65 \pm 6$; DAPA/EXE, $\Delta = 46 \pm 2$) and lower EGP decrease in DAPA ($\Delta = -0.66 \pm 0.04$ mg/kg/min) vs EXE ($\Delta = -0.84 \pm 0.05$) were also comparable; in DAPA/EXE ($\Delta = -0.65 \pm 0.03$) it was equal to DAPA and lower than EXE. Changes in plasma insulin/glucagon could not explain higher EGP in DAPA/EXE vs EXE mg/kg/min.

Conclusion: Our findings provide strong evidence for the emergence of a new long-lasting, glucose-independent, insulin/glucagon-independent, glucoregulatory mechanism via which SGLT2i-induced glycosuria stimulates EGP in patients with T2D. SGLT2i plus GLP-1 receptor agonist combination therapy is accompanied by superior glycemic control vs monotherapy.

Key Words: endogenous glucose production, glucose regulation, GLP-1 receptor agonists, kidney, SGLT-2 inhibitors, type 2 diabetes

Abbreviations: ANOVA, analysis of variance; DAPA, dapagliflozin; DPP4i, dipeptidyl peptidase 4 inhibitor; EGP, endogenous glucose production; EXE, exenatide; FFA, free fatty acid; GLP-1, glucagon-like peptide 1; INS/GCN, insulin to glucagon; OGTT, oral glucose tolerance test; PG, plasma glucose; RA, receptor agonist; SGLT2i, sodium–glucose cotransporter 2 inhibitor; UGE, urinary glucose excretion.

Sodium–glucose cotransporter 2 inhibitors (SGLT2is) reduce plasma glucose (PG) by producing glycosuria (1-3), but their glucose-lowering effect is partially offset by an increase in endogenous glucose production (EGP) (2-4), which compensates for ~50% the amount of glucose excreted in urine (2, 5). In response to acute (3) and chronic (4) SGLT2i administration, the fasting plasma glucagon concentration increases and plasma insulin concentration decreases. However, these hormonal changes cannot explain the rise in basal EGP, which persists when plasma glucagon and insulin are clamped at fasting levels (6). Further, the increase in basal EGP is not abolished when an SGLT2i is administered with a glucagon-like peptide 1 (GLP-1) receptor agonist (RA) or a dipeptidyl peptidase 4 inhibitor (DPP4i), which increase plasma insulin and decrease plasma glucagon (7, 8). Recently, we demonstrated that, following a single dose of SGLT2i, suppression of EGP is impaired after an oral glucose load. This was evident even when the oral glucose challenge was administered with exenatide (EXE), which augmented insulin and suppressed glucagon secretion (9). Although the triggering mechanism(s) responsible for the SGLT2i-induced stimulation of EGP have yet to be completely elucidated, activation of the renal nerves by

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glycosuria (10-12) is believed to play a role, by transmitting a neuroendocrine signal to the liver to stimulate hepatic glucose output.

All previous studies which examined the effect of SGLT2i on EGP have been carried out during acute exposure to a single dose of drug (3, 6, 9, 11, 12), and long-term (months) changes in EGP after SGLT2i therapy only have been examined in a few studies under fasting conditions (4, 7, 8). A sequential analysis including initial and chronic effects of SGLT2is on EGP and glucose kinetics has not been reported. In the present study, we utilized a double tracer oral glucose tolerance test (OGTT) technique (9, 13, 14) to examine long-term changes in glucose kinetics in patients with type 2 diabetes treated with dapagliflozin (DAPA) and compared the results with those in patients treated with the GLP-1 RA EXE plus DAPA, to augment insulin and suppress glucagon secretion. Our hypothesis was that increased EGP in response to acute SGLT2i-induced glycosuria persists for a long period of time and is not abolished by GLP-1 RA stimulation of insulin secretion and glucagon suppression. Moreover, the enhancement of EGP during glycosuria also is not affected by an oral glucose load. If confirmed, these findings will establish a new glucose and insulin/glucagon-independent glucoregulatory mechanism for glycemic control in patients with type 2 diabetes treated with an SGLT2i.

Table 1. Baseline characteristics of study subjects

Characteristics	Dapagliflozin	Exenatide	Dapagliflozin + exenatide
Number	25	25	25
Age (years)	52 ± 3	51 ± 2	49 ± 2
Sex (M/F)	10 M/15F	13 M/12F	9 M/16F
Weight (kg)	85.2 ± 3.1	93.2 ± 3.7	92.1 ± 2.5
BMI (kg/m^2)	32.5 ± 1.1	32.6 ± 1.2	32.7 ± 0.9
HbA1c (%)	8.3 ± 0.2	8.0 ± 0.2	8.4 ± 0.3
HbA1c (mmol/mol)	66.3 ± 2.4	63.9 ± 2.4	67.1 ± 2.6
Diabetes duration (years)	7.7 ± 1.9	6.6 ± 1.6	6.9 ± 2.1
FPG (mg/dL)	159 ± 10	160 ± 7	177 ± 11
Plasma creatinine (mg/dL)	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
eGFR (mL/min/1.73 m^2)	101 ± 6	98 ± 4	102 ± 2
Treatment			
Diet	5	4	5
Metformin	12	10	11
Metformin/SU	8	11	9

Data are presented as mean ± SD, or n.
Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; SU, sulfonylurea.

Materials and Methods

Subjects

Seventy-five subjects with type 2 diabetes participated in the study during the period December 2017 to June 2022. Baseline treatment included diet (n = 15), stable (>3 months) metformin dose (n = 41), and metformin plus sulfonylurea (n = 31). Except for diabetes, all subjects were in good general health based on medical history, physical examination, screening blood tests, urinalysis, and electrocardiogram. Clinical, anthropometric and laboratory data at baseline were comparable between groups and are shown in Table 1. Body weight was stable (±1.5 kg) for at least 3 months prior to study, and no subject participated in any excessively heavy exercise program. Subjects taking drugs known to affect glucose metabolism (other than metformin and sulfonylurea) were excluded. The study was approved by University of Texas Health San Antonio IRB, and informed written consent was obtained from all participants.

Subjects participated in 3 double tracer OGTT studies (9, 14) that were performed at baseline after a single dose (acute study) of medication (DAPA, n = 25; EXE, n = 25; DAPA + EXE, n = 25) and after 1 month and 4 months of drug treatment.

Experimental Design

All studies were performed on the Clinical Research Center (CRC) of the Texas Diabetes Institute at 07:00 hours following an overnight fast. During double tracer OGTT subjects received 8-hour prime (40 µCi × FPG/100) continuous (0.40 µCi/min) 3-^{3}H-glucose infusion via an antecubital vein catheter, as previously described (9). After a 3-hour tracer equilibration period, subjects ingested 75 g of glucose (Trutol 75, Fischer Scientific, Middletown, VA, USA) containing 100 µCi of 1-^{14}C-glucose (Perkin Elmer, Boston, MA, USA). Thereafter, subjects received (1) oral DAPA 10 mg; (2) EXE 5 µg subcutaneously; or (3) DAPA 10 mg plus EXE 5 µg (DAPA + EXE), in random order. Urine was collected prior to and after drug administration for measurement of urinary glucose excretion. Following completion of the double tracer OGTT, subjects were given 1 of their assigned medications and instructed to consume a weight-maintaining diet for 4 months (Fig. 1). They were asked to continue with their metformin or metformin/sulfonylurea. Patients were contacted weekly by phone and returned to CRC every 2 to 4 weeks. Arterialized venous blood samples ("hot box" technique) were drawn from a hand vein at −30, −20, −10, −5, 0, and every 15 to 20 minutes thereafter for 5 hours for PG, insulin, C-peptide, and glucagon concentrations, and tritiated glucose and ^{14}C-glucose radioactivity. Plasma insulin was determined with immunoradiometric assay (BioZ, Louvain, Belgium) using a double-antibody assay (RRID:AB_10575430 and RRID:AB_2233795); C-peptide was determined using a double-antibody immunoassay (RRID:AB_1614416 and RRID:AB_1614415); and glucagon was determined using a single antibody immunoassay (RRID:AB_2757819) (both radioimmunoassay from EMD Millipore, Billerica, MA, USA). Fluorometric assays were used to measure plasma free fatty acids (FFAs) (intra-assay coefficient of variation [CV] 4.91%, interassay CV 0.75%; FUJIFILM Wako Diagnostics, Richmond, VA, USA). At month 1 and month 4 the same double tracer OGTT protocol was followed. After the OGTT at month 1, subjects receiving EXE 5 µg twice a day and those in the DAPA + EXE groups were switched to EXE 2 mg/week injections. At month 4, all patients switched to EXE 2 mg weekly received an OGTT 4 days after the last dose of weekly EXE.

Data Analysis

The primary end point was change in EGP from baseline (−30 to 0 minutes) to the 300-minute time period of OGTT

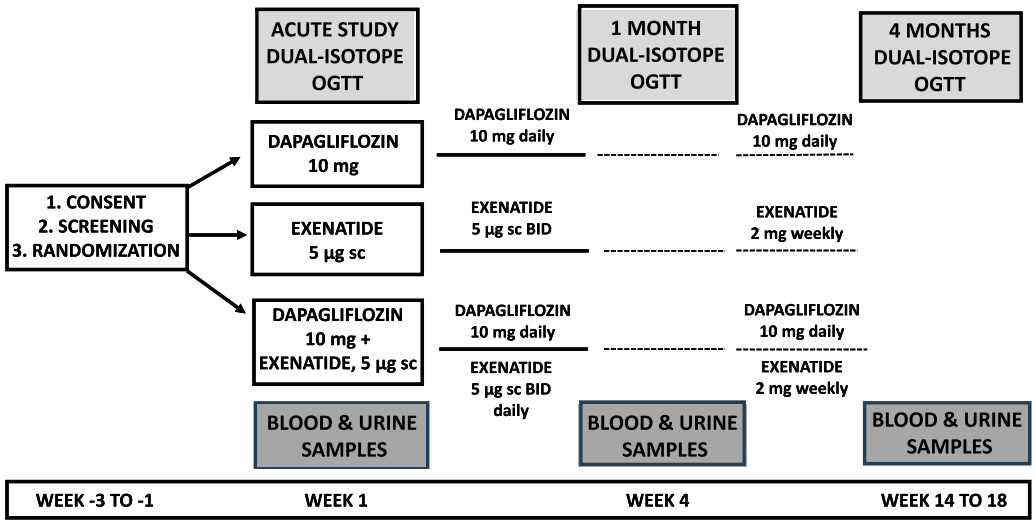

Figure 1. Schematic diagram of the study design. Dual-isotope refers to 3-H^3-glucose intravenous infusion plus oral ^{14}C-glucose intake; OGTT = oral glucose tolerance test; blood and urine samples were collected during the OGTT procedures performed in all patients after a single drug administration (acute) and 1 month and 4 months of therapy with each study drug used alone or in combination.

following drug administration. Under steady-state postabsorptive conditions, the basal rate of EGP equals the (3-^{3}H)-glucose infusion rate divided by steady-state plasma tritiated glucose specific activity (15). After drug administration, nonsteady conditions prevail, and rates of total body glucose appearance (Ra) and disappearance were calculated using Steele's equation (15). Rates of oral glucose appearance (RaO) and endogenous glucose production were calculated as previously described (9, 14). Splanchnic (hepatic plus gastrointestinal tissues) glucose uptake was calculated as the difference between the ingested glucose (75 g) and RaO.

Change in EGP (and all glucose kinetic parameters, hormone/metabolite concentrations) from baseline to the mean of the 300-minute OGTT period after each drug administration (acute vs 1 month vs 4 months) was compared with analysis of variance (ANOVA). Change in EGP from −30 to 0 minutes vs mean EGP from 0 to 300 minutes following each individual drug administration was compared using the paired t test. Post hoc testing was done using Bonferroni correction. Values are presented as mean ± SEM. $P < .05$ was considered to be statistically significant.

Results

Plasma Glucose Concentration

During the acute study with DAPA, PG increased from 152 ± 9 to 225 ± 12 mg/dL (Δ = +72 ± 3 mg/dL) during the 300-minute period of the OGTT, which was significantly higher ($P < .01$) than both EXE (152 ± 7 to 194 ± 7; Δ = +42 ± 1 mg/dL) and DAPA + EXE (182 ± 10 to 191 ± 13; Δ = +9 ± 3 mg/dL); the increase in PG with DAPA/EXE was also lower ($P < .05$) than EXE (Fig. 2A).

At 1 month, fasting PG in DAPA, EXE, and DAPA + EXE was similar and significantly reduced ($P < .01$) vs the pretreatment value. During the 0- to 300-minute period of the OGTT, the increment in PG in DAPA + EXE (Δ = +27 ± 1 mg/dL) was similar to EXE (Δ = +26 ± 1 mg/dL) and both were significantly ($P < .01$) lower than DAPA (Δ = +62 ± 2 mg/dL) (Fig. 2B).

At 4 months, fasting PG was lower ($P < .01$) in DAPA + EXE vs DAPA and EXE. During the 0- to 300-minute time period of the OGTT, the PG increment in DAPA (Δ = +65 ± 6 mg/dL) was higher ($P < .05$) than EXE (Δ = +55 ± 2 mg/dL), which was higher ($P < .05$) than DAPA + EXE (Δ = +46 ± 2 mg/dL) (Fig. 2C).

Endogenous Glucose Production

During the acute study, basal rate of EGP (2.27 ± 0.11) decreased to a mean of 1.62 ± 0.09 mg/kg/min (Δ = −0.65 ± 0.03 mg/kg/min) with DAPA, which was attenuated ($P < .01$) when compared with EXE (2.33 ± 0.13 to 1.37 ± 0.06 mg/kg/min, Δ = −0.96 ± 0.07). In DAPA + EXE the decrease in EGP (2.41 ± 0.10 to 1.56 ± 0.10 mg/kg/min, Δ = −0.84 ± 0.05) was significantly less than EXE ($P < .05$) (Fig. 2D). EGP suppression during the OGTT was 28% in DAPA, 41% in EXE and intermediate, 35% in DAPA + EXE.

After 1 month, baseline EGP in DAPA and DAPA + EXE (Fig. 2E; Table 2) were similar to the acute study, despite lower fasting PG (Fig. 2A and 2B). In EXE, baseline EGP was lower ($P < .05$) at 1 month than the acute study and also lower than DAPA or DAPA + EXE (Fig. 2E and Table 2). After 1 month, the pattern of change in EGP during the OGTT was similar to that in the acute study in all 3 groups. In DAPA, mean EGP (0-360 minutes OGTT) decreased from 2.47 ± 0.14 to 1.87 ± 0.09 mg/kg/min (Δ = −0.60 ± 0.05). After 1 month, in EXE, the decrease in EGP (2.10 ± 0.09 to 1.33 ± 0.10, Δ = −0.77 ± 0.04) tended to be lower than DAPA + EXE (2.41 ± 0.10 to 1.57 ± 0.06, Δ = −0.72 ± 0.03), but the difference did not reach statistical significance. Percent suppression of EGP after 1 month was 24% in DAPA, 37% in EXE, and 32% in DAPA + EXE.

After 4 months, baseline EGP in DAPA (2.41 ± 0.06 mg/kg/min) was higher ($P < .05$) than EXE (2.25 ± 0.07 mg/kg/min) and DAPA + EXE (2.23 ± 0.06 mg/kg/min), and similar to the acute and 1-month studies (Table 2). After 4 months, EGP suppression during OGTT was similar to the acute and 1-month studies in all 3 groups. Suppression of EGP during OGTT with DAPA + EXE (to 1.58 ± 0.06 mg/kg/min) was also significantly

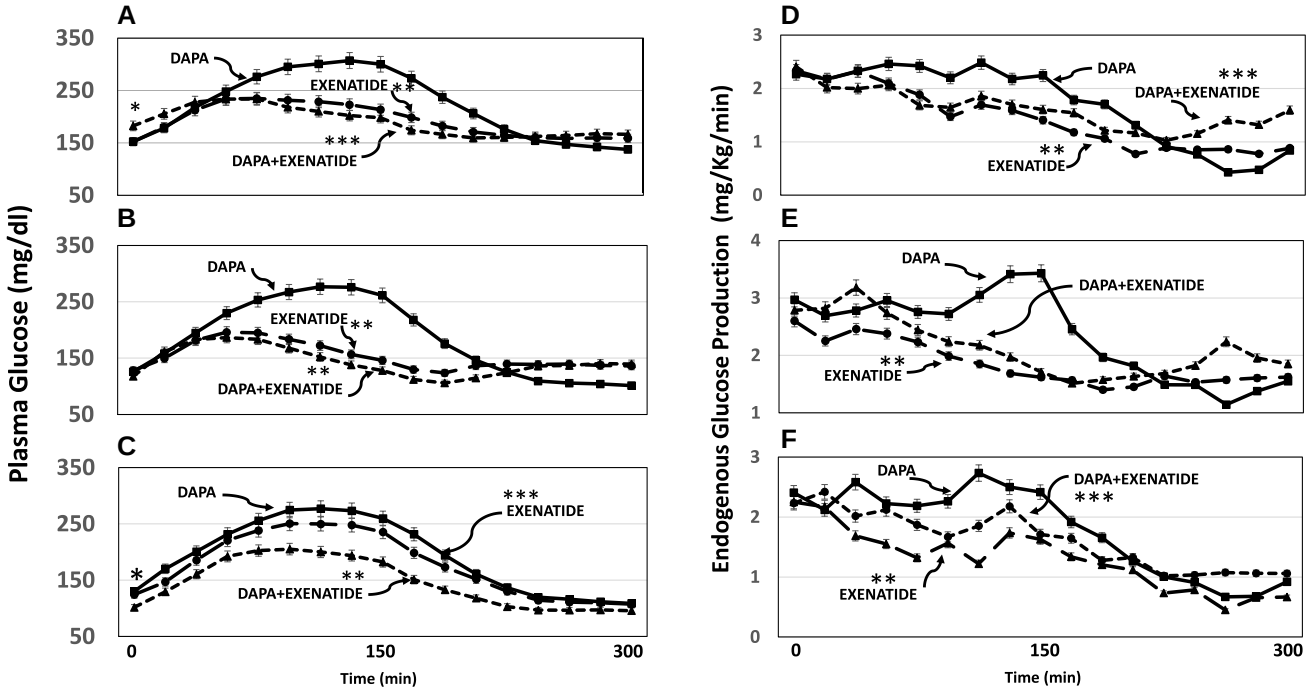

Figure 2. (A-C) Plasma glucose concentration (PG) during 300-minute, 75-g oral glucose tolerance test (OGTT) after acute (single dose) administration of dapagliflozin (DAPA), exenatide (EXE), and EXE plus DAPA (DAPA + EXE) (Fig. 1A) and after administration of DAPA, EXE, and DAPA + EXE for 1 month (Fig. 1B) and 4 months (Fig. 1C). (D-F) Endogenous glucose production (EGP) during the 300-minute, 75-g OGTT after acute (single dose) administration of DAPA, EXE, and DAPA + EXE (Fig. 1D) and after administration of DAPA, EXE , and DAPA + EXE for 1 month (Fig. 1E) and 4 months (Fig. 1F). *$P < .05$, baseline differences between groups; **$P < .01$, mean OGTT values between groups; ***$P < .01$, mean OGTT values between EXE vs DAPA + EXE (ANOVA alone).

Table 2. Rates of total glucose appearance, endogenous glucose production, and oral glucose appearance at baseline and during oral glucose tolerance test in all 3 groups in acute studies and after 1 month and 4 months of therapy

	Total Ra (mg/kg/min)		EGP (mg/kg/min)		RaO (mg/kg/min)	
	Baseline	OGTT	Baseline	OGTT	Baseline	OGTT
Acute study						
DAPA	2.27 ± 0.04	4.15 ± 0.17[a]	2.27 ± 0.04	1.62 ± 0.10[a]	—	2.53 ± 0.11[a]
EXE	2.33 ± 0.13	3.38 ± 0.13	2.33 ± 0.13	1.37 ± 0.06[b]	—	2.01 ± 0.09
DAPA + EXE	2.41 ± 0.10	3.51 ± 0.09	2.41 ± 0.05	1.56 ± 0.07	—	1.95 ± 0.12
1-month study						
DAPA	2.47 ± 0.05[a]	4.58 ± 0.26[a]	2.47 ± 0.05[a]	1.87 ± 0.10[a]	—	2.71 ± 0.16[a]
EXE	2.10 ± 0.09	3.15 ± 0.18	2.10 ± 0.09	1.33 ± 0.10[b]	—	1.82 ± 0.16
DAPA + EXE	2.29 ± 0.05	3.48 ± 0.14	2.29 ± 0.05	1.57 ± 0.06	—	1.91 ± 0.17
4-month study						
DAPA	2.41 ± 0.06[a]	4.46 ± 0.26[a]	2.41 ± 0.06[a]	1.75 ± 0.10[a]	—	2.71 ± 0.16[a]
EXE	2.25 ± 0.13	3.56 ± 0.16[b]	2.25 ± 0.07	1.41 ± 0.08[b]	—	2.15 ± 0.10
DAPA + EXE	2.23 ± 0.06	3.90 ± 0.09	2.23 ± 0.06	1.58 ± 0.06	—	2.32 ± 0.11

Abbreviations: DAPA, dapagliflozin; EGP, endogenous glucose production; EXE, exenatide; OGTT, oral glucose tolerance test; RaO, rate of oral glucose appearance in peripheral circulation; Total Ra, rate of total glucose appearance.
[a]$P < .05$, DAPA vs EXE and DAPA + EXE.
[b]$P < .05$, EXE vs DAPA + EXE (all P values were derived from analysis of variance with Bonferroni's post hoc testing).

less ($P < .01$) than with EXE (to 1.41 ± 0.08 mg/kg/min) (Fig. 2F and Table 2). At month 4, the decrement in EGP during OGTT with DAPA ($\Delta = -0.66 \pm 0.04$ mg/kg/min) and DAPA + EXE ($\Delta = -0.65 \pm 0.03$) were comparable and significantly lower ($P < .01$) than EXE ($\Delta = -0.84 \pm 0.05$). Percent EGP suppression during the OGTT after 4 months was similar to that during the acute and 1-month studies: 27% in DAPA, 37% in EXE, and 30% in DAPA + EXE.

Plasma Free Fatty Acid Concentration

In the acute study, plasma FFAs decreased during the 300-minute period of the OGTT from a baseline fasting value of 550 ± 30 to a mean of 230 ± 20 µmol/L in DAPA, from 590 ± 54 to 260 ± 22 in EXE, and from 690 ± 33 to a mean of 300 ± 350 in DAPA/EXE. At 1 month, fasting plasma FFAs in DAPA decreased during the OGTT from 620 ± 40 to 290 ± 20 µmol/L, in EXE from 480 ± 36 to 230 ± 20, and in DAPA/EXE from 600 ± 45 to 250 ± 28. At 4 months, fasting plasma FFA decreased from 720 ± 45 to 290 ± 28 µmol/L during the OGTT in DAPA, from 480 ± 36 to 230 ± 20 in EXE, and from 670 ± 36 to 260 ± 20 µmol/L in DAPA/EXE (all $P < .001$). In DAPA, there was a significant rise in baseline fasting plasma FFAs after 1 month (Δ = +70 ± 24 µmol/L) and 4 months (Δ = +100 ± 32 µmol/L) compared with acute (both $P < .05$), whereas in EXE fasting plasma FFA decreased at 1 month (Δ = −110 ± 25 µmol/L) and at 4 months (Δ = −92 ± 36 µmol/L) (both $P < .05$). In DAPA/EXE there was a lesser decrease in fasting plasma FFA both at 1 month (Δ = −52 ± 20 µmol/L) and at 4 months (Δ = −12 ± 18 µmol/L) ($P < .05$ vs DAPA).

Plasma Hormones

During acute study, the rise in plasma insulin during the OGTT was similar in DAPA and DAPA + EXE groups and significantly higher ($P < .01$) with EXE (Fig. 3A). During the 1-month and 4-month studies, plasma insulin response during the OGTT with EXE was increased ~3- to 4-fold compared with the acute study ($P < .01$) and was significantly increased compared with DAPA + EXE (Fig. 3A-3C). Similar patterns and directional changes in plasma C-peptide to those of insulin were documented in all studies (Fig. 3D-3F).

In the acute study, plasma glucagon during OGTT decreased slightly in EXE and DAPA + EXE groups, whereas in DAPA it did not change ($P < .05$ vs all). A similar pattern was observed after 1 month and 4 months, with no change in plasma glucagon in the DAPA group and significant ($P < .01$, OGTT vs baseline at 1 month) decreases in plasma glucagon in the EXE group ($P < .05$, DAPA vs ALL). In the DAPA + EXE group, plasma glucagon tended to decline (P = NS) during the OGTT (Fig. 3G–I).

During the acute study, insulin to glucagon (INS/GCN) ratio increased from 0.26 ± 0.02 (baseline) to 0.52 ± 0.05 µU/mL per pg/mL (Δ = +0.26) during the 300-minute OGTT with DAPA, whereas with EXE, the INS/GCN ratio increased ~4-fold from baseline (0.29 ± 0.02 to 1.10 ± 0.03, Δ = +0.81) ($P < .001$ vs DAPA). With DAPA + EXE, the increase in the INS/GCN ratio during OGTT was attenuated (0.30 ± 0.03 to 0.72 ± 0.05, Δ = +0.42) ($P < .01$, Δ INS/GCN ratio, EXE vs DAPA and DAPA + EXE). At month 1, the rise in the INS/GCN ratio in DAPA (0.27 ± 0.03 to 0.64 ± 0.06, Δ = +0.37) during OGTT was similar to the acute study and was significantly ($P < .001$) lower than in EXE (0.31 ± 0.05 to 1.83 ± 0.08, Δ = +1.52) and DAPA + EXE (0.32 ± 0.04 to 0.97 ± 0.06, Δ = +0.65). A similar pattern was seen at month 4 during the OGTT, with a lower ($P < .001$) increase in the INS/GCN ratio in DAPA (0.21 ± 0.04 to 0.55 ± 0.05, Δ = +0.34) vs EXE (0.36 ± 0.07 to 1.79 ± 0.10, Δ = +1.43) and DAPA + EXE (0.27 ± 0.05 to 1.03 ± 0.08, Δ = +0.76). In the acute 1-month and 4-month studies, the INS/GCN ratio during OGTT in DAPA + EXE was significantly lower ($P < .05$) than with EXE and higher than in DAPA.

The decrement in EGP from baseline to a mean value during the OGTT was lower in DAPA than in EXE ($P < .05$) and intermediary in DAPA + EXE ($P < .05$ vs EXE) in the acute study and after 1 month and 4 months (Fig. 4A). The elevation in plasma insulin concentration from baseline to a mean value during the OGTT was lower in DAPA and DAPA + EXE than in EXE ($P < .05$) in all 3 studies (Fig. 4B). The decrease in plasma glucagon concentration during the OGTT was attenuated in DAPA ($P < .05$) compared with EXE and DAPA + EXE in the acute study and after 1 month and 4 months (Fig. 4C).

Glucose Kinetics

During acute study, the rate of oral glucose appearance in the systemic circulation (RaO) during the 300-minute OGTT was higher ($P < .05$) in DAPA (2.53 ± 0.11 mg/kg/min) than EXE (2.01 ± 0.09) and DAPA + EXE (1.95 ± 0.12). During the 1-month and 4-month studies, RaO during the 300-minute OGTT followed a similar pattern to the acute study in all 3 groups.

The rate of total glucose disappearance (total Rd) during the OGTT in the acute study increased from baseline of 2.27 ± 0.04 to 3.98 ± 0.18 mg/kg/min (Δ = +1.71 ± 0.11) with DAPA, which was significantly greater than EXE (Δ = +1.04 ± 0.12 mg/kg/min) and DAPA + EXE (Δ = +1.07 ± 0.14 mg/kg/min). Urinary glucose excretion (UGE) increased in DAPA from 0.03 ± 0.01 at baseline to 0.98 ± 0.15 mg/kg/min during the 300-minute OGTT ($P < .001$ vs baseline). This increase was similar to that with DAPA + EXE (Δ = +0.92 ± 0.07 mg/kg/min); with EXE, UGE increased slightly (0.03 ± 0.01 vs 0.17 ± 0.05 mg/kg/min) (Table 3). There was a comparable increase in tissue glucose disappearance (tissue Rd) during the 300-minute OGTT in EXE (2.30 ± 0.12 vs 3.20 ± 0.05 mg/kg/min) and DAPA (2.24 ± 0.07 to 3.00 ± 0.13) (both $P < .05$ vs baseline); the increase in tissue Rd in DAPA + EXE (2.33 ± 0.07 to 2.48 ± 0.06) was not statistically significant. There were no significant changes in tissue metabolic clearance rates of glucose (tissue MCR_G) with any of the treatments (Table 3).

At 1 month, RaO during the 300-minute OGTT was significantly ($P < .01$) higher with DAPA vs both EXE and EXE/DAPA (Table 3). During the OGTT, UGE increased similarly in DAPA (0.51 ± 0.08 to 1.11 ± 0.07 mg/kg/min) and DAPA/EXE (0.30 ± 0.08 to 0.82 ± 0.12) and did not change with EXE (0.03 ± 0.02 to 0.05 ± 0.02) ($P < .001$ vs DAPA and DAPA + EXE). The increase in tissue Rd during the 300-minute OGTT at 1 month was comparable between DAPA (2.99 ± 0.09 mg/kg/min) and EXE (3.11 ± 0.16 mg/kg/min) and higher ($P < .05$) than DAPA + EXE (2.66 ± 0.06 mg/kg/min). Tissue MCR_G during the OGTT tended to increase in DAPA (1.89 ± 0.15 to 2.10 ± 0.19 mL/kg/min) and EXE (1.70 ± 0.12 to 2.07 ± 0.11 mL/kg/min) (both P = NS) and did not change with DAPA + EXE (1.89 ± 0.11 vs 1.87 ± 0.13 mL/kg/min) (Table 3). UGE increased in DAPA from 0.51 ± 0.08 at baseline to 1.11 ± 0.07 mg/kg/min during the 300-minute OGTT ($P < .001$). This increase was similar to that with DAPA + EXE (Δ = +0.52 ± 0.04 mg/kg/min); with EXE, UGE did not change (0.03 ± 0.02 vs 0.05 ± 0.02 mg/kg/min) (Table 3).

At 4 months, RaO and UGE paralleled those at 1 month in all 3 groups (DAPA, EXE, and DAPA + EXE) (Table 3). Tissue Rd increased similarly in DAPA (1.80 ± 0.06 to 2.74 ± 0.16 mg/kg/min) and DAPA + EXE (2.03 ± 0.06 to

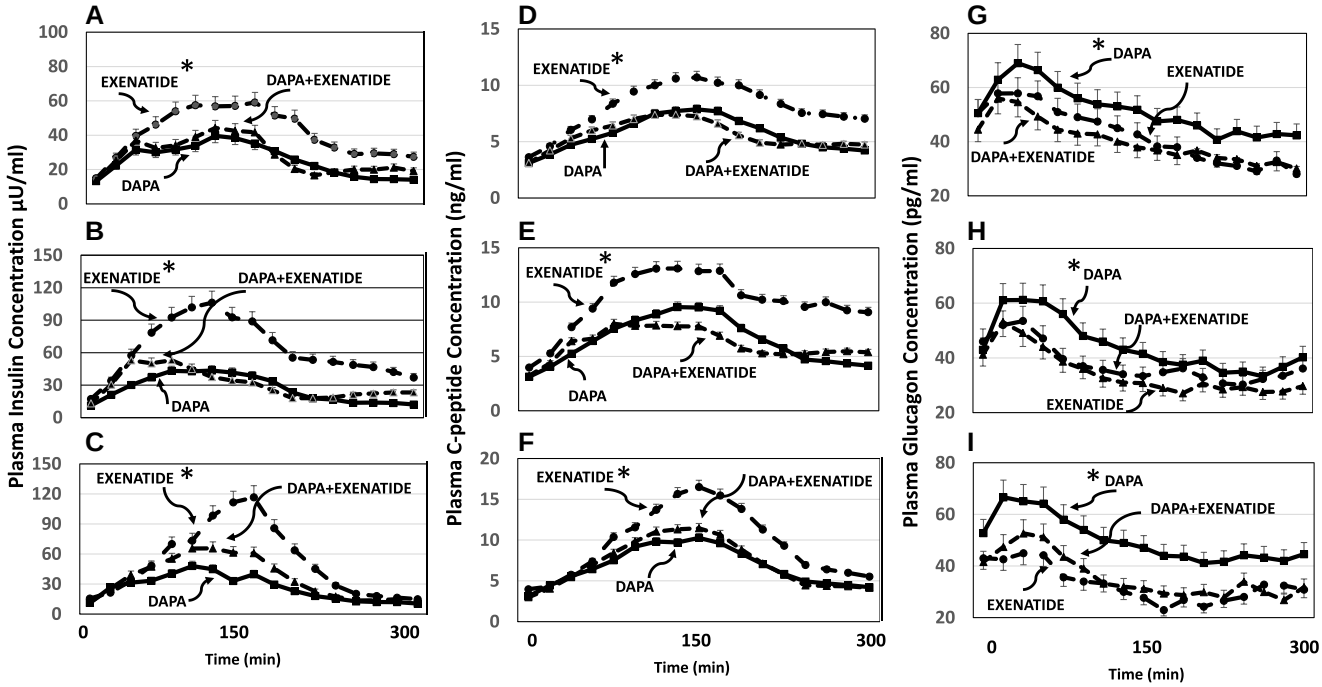

Figure 3. (A-C) Plasma insulin concentration during the 300-minute, 75-g oral glucose tolerance test (OGTT) after acute (single dose) administration of dapagliflozin (DAPA), exenatide (EXE), and EXE plus DAPA (DAPA + EXE) (A) and, after therapy with DAPA, EXE, and DAPA + EXE for 1 month (B) and 4 months (C). *$P < .05$ EXE vs DAPA and DAPA + EXE (ANOVA). (D-F) Plasma C-peptide concentration during the 300-minute, 75-g oral glucose tolerance test (OGTT) after acute (single dose) administration of DAPA, EXE, and DAPA + EXE) (D) and,- after therapy with DAPA, EXE, and DAPA + EXE for 1 month (E) and 4 months (F). *$P < .05$ EXE vs DAPA and DAPA + EXE.- (ANOVA). (G-I) Plasma glucagon concentration during the 300-minute, 75-g oral glucose tolerance test (OGTT) after acute (single dose) administration of DAPA, EXE, and DAPA + EXE (G) and after therapy with DAPA, EXE, and DAPA + EXE for 1 month (H) and 4 months (I). *$P < .05$ DAPA vs EXE and DAPA + EXE (ANOVA).

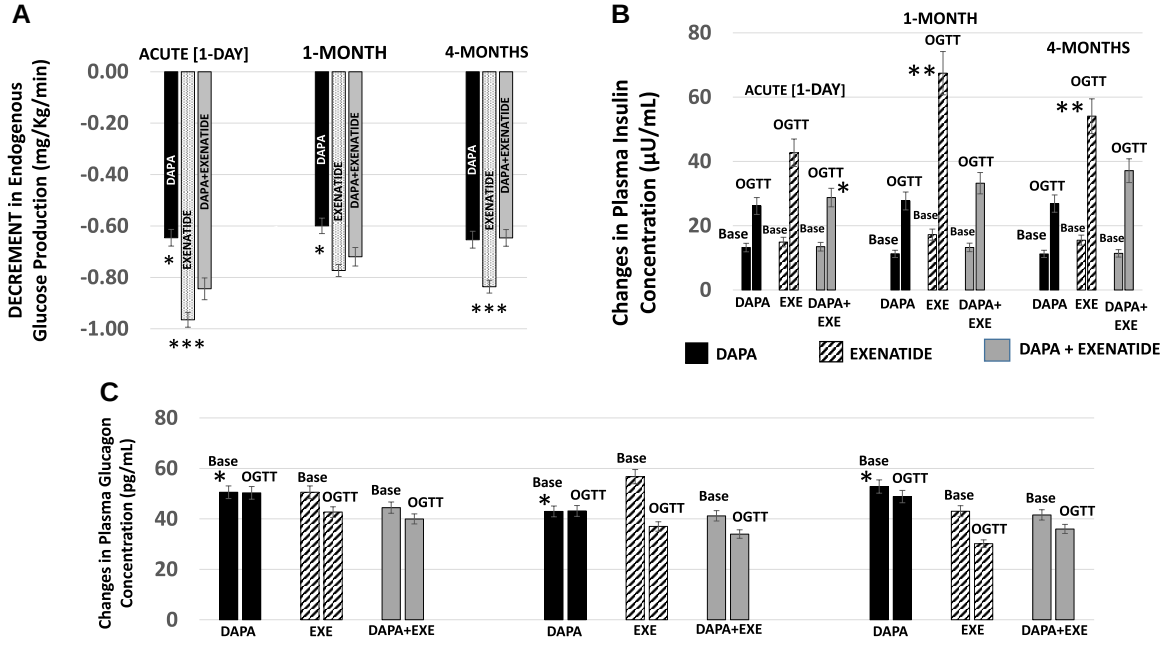

Figure 4. (A) Decrement in EGP (baseline minus mean value during the 300-minute period of oral glucose tolerance test (OGTT) after acute (single dose) administration dapagliflozin (DAPA), exenatide (EXE), and DAPA + EXE and after administration of DAPA, EXE, and DAPA + EXE for 1 month and 4 months. Data represent mean ± SEM. (B) Changes in plasma insulin concentration between baseline (BASE) and the mean value during the 300-minute time period of OGTT, preceded by the administration of a single dose of DAPA, EXE, and DAPA + EXE in the acute (1-day) study and after 1 month and 4 months of therapy with DAPA, EXE, DAPA + EXE. Data represent mean ± SEM. (C) Changes in plasma glucagon concentration between baseline (BASE) and the mean value during the 300-minute time period of OGTT, preceded by the administration of a single dose of DAPA, EXE, and DAPA + EXE in the acute (1-day) study and after 1 month and 4 months of therapy with DAPA, EXE, DAPA + EXE. Data represent the mean ± SEM. *$P < .05$, increment/decrement differences between all groups; **$P < .01$, increment/decrement differences between all groups; ***$P < .05$, EXE vs DAPA + EXE. All P values were derived from ANOVA with Bonferroni's post hoc testing.

Table 3. Rates of total glucose disappearance, urinary glucose excretion, tissue glucose disappearance, and metabolic clearance of glucose at baseline and during oral glucose tolerance test during acute study and after 1 month and 4 months of therapy

	Total Rd		Total Rd (mg/kg/min)		UGE (mg/kg/min)		Tissue Rd (mg/kg/min)	
	Baseline	OGTT	Baseline	OGTT	Baseline	OGTT	Baseline	OGTT
Acute study								
DAPA	2.27 ± 0.04	3.98 ± 0.18[a]	0.03 ± 0.01	0.98 ± 0.15	2.24 ± 0.07	3.00 ± 0.13	1.48 ± 0.09	1.29 ± 0.07
EXE	2.33 ± 0.13	3.37 ± 0.10	0.03 ± 0.01	0.17 ± 0.05[c]	2.30 ± 0.12	3.20 ± 0.05[d]	1.57 ± 0.10[d]	1.69 ± 0.09[d]
DAPA + EXE	2.41 ± 0.10	3.48 ± 0.18	0.08 ± 0.03	1.00 ± 0.12	2.33 ± 0.07	2.48 ± 0.06	1.28 ± 0.10	1.29 ± 0.12
1-month study								
DAPA	2.47 ± 0.05[a]	4.10 ± 0.16[a]	0.51 ± 0.08	1.11 ± 0.07	1.96 ± 0.03	2.99 ± 0.09	1.89 ± 0.15	2.10 ± 0.19
EXE	2.10 ± 0.09	3.16 ± 0.18	0.03 ± 0.02[c]	0.05 ± 0.02[c]	2.07 ± 0.07	3.11 ± 0.16	1.70 ± 0.12	2.07 ± 0.11[b]
DAPA + EXE	2.29 ± 0.05	3.48 ± 0.15	0.30 ± 0.08	0.82 ± 0.12	1.99 ± 0.05	2.66 ± 0.06[e]	1.89 ± 0.11	1.87 ± 0.13
4-month study								
DAPA	2.41 ± 0.06[a]	4.32 ± 0.24[a]	0.61 ± 0.06	1.58 ± 0.08	1.80 ± 0.06	2.74 ± 0.16	1.78 ± 0.12	1.97 ± 0.19
EXENATIDE	2.25 ± 0.13	3.51 ± 0.15	0.03 ± 0.01[c]	0.14 ± 0.03[c]	2.22 ± 0.12[d]	3.37 ± 0.12[d]	1.91 ± 0.15	2.01 ± 0.11
DAPA + EXENATIDE	2.23 ± 0.06	3.87 ± 0.10	0.20 ± 0.06	1.20 ± 0.16	2.03 ± 0.06	2.67 ± 0.06	2.06 ± 0.13	1.83 ± 0.16

Abbreviations: DAPA, dapagliflozin; EXE, exenatide; MCR_G, tissue metabolic clearance rate of glucose (tissue Rd/plasma glucose concentration); OGTT, oral glucose tolerance test; Tissue Rd, (total Rd – UGE); Total Rd, rate of total body glucose disappearance; UGE, urinary glucose excretion.
[a]$P < .05$, DAPA vs EXE and DAPA + EXE.
[b]$P < .05$, EXE vs DAPA + EXE.
[c]$P < .001$ EXE vs DAPA and DAPA + EXE.
[d]$P < .05$, EXE vs DAPA and DAPA + EXE.
[e]$P < .05$, DAPA + EXE vs DAPA and EXE (all P values were derived from analysis of variance with Bonferroni's post hoc testing).

2.67 ± 0.06 mg/kg/min), but the increments were lower than EXE (2.22 ± 0.12 to 3.37 ± 0.12 mg/kg/min, $P < .05$). There were small increases in tissue MCR_G in DAPA (1.78 ± 0.12 to 1.97 ± 0.19 mL/kg/min) and EXE (1.91 ± 0.15 to 2.01 ± 0.1 mL/kg/min), and a small decrease in DAPA + EXE (2.06 ± 0.13 vs 1.83 ± 0.16) (all P = NS) (Table 3).

In the acute study, during the 300-minute OGTT 106.9 ± 3.8 g of glucose appeared in the peripheral circulation (total Ra) following DAPA. Of these, 65.2 ± 2.8 g were derived from oral glucose load (RaO) and 41.7 ± 1.6 g from EGP. During the same period, 25.3 ± 3.4 g were excreted in urine. In EXE, Total Ra was 92.4 ± 4.8 g; 54.9 ± 2.8 g were derived from the oral glucose load and 37.5 ± 3.4 g from EGP ($P < .05$ vs DAPA); 4.7 ± 0.5 g were excreted in urine. In DAPA + EXE, total Ra was 93.9 ± 3.4 g, of which 52.2 ± 3.4 g were derived from RaO and 41.7 ± 1.8 g from EGP ($P < .05$ vs EXE); during this period, 27.8 ± 2.3 g were excreted in urine.

At 1 month, total Ra over the 300-minute OGTT was 118.1 ± 4.9 g in DAPA, of which RaO accounted for 69.8 ± 2.8 g and EGP for 48.3 ± 3.4 g ($P < .01$ vs DAPA during acute study); during the same period, 28.6 ± 1.6 g of glucose were excreted in urine. Total Ra in EXE was 86.1 ± 3.6 g; 49.7 ± 2.3 g were derived from RaO and 36.4 ± 1.9 g from EGP ($P < .01$ vs DAPA); urinary glucose excretion was 1.3 ± 0.5 g. In DAPA + EXE, total Ra was 93.1 ± 3.4 g, with 51.1 ± 1.8 g from RaO and 37.5 ± 1.7 g from EGP; 21.9 ± 1.5 g were lost in urine.

At 4 months, total Ra over the 300-minute OGTT was 114.9 ± 5.2 g in DAPA, with 69.7 ± 4.8 g accounted for by RaO and 45.2 ± 1.7 g by EGP; during this period, 40.7 ± 2.1 g of glucose were excreted in urine. In EXE, Total Ra amounted to 97.3 ± 2.9 g, of which 58.8 ± 3.2 g derived from RaO and 38.5 ± 1.8 g from EGP; urinary glucose excretion was 3.8 ± 0.7 g. Total Ra in DAPA + EXE was 104.4 ± 7.3 g, of which 62.1 ± 3.1 g were derived from RaO and 42.3 ± 2.7 g from EGP ($P < .05$ vs EXE); during this period, 32.1 ± 2.4 g were excreted in urine.

Clinical Outcomes and Adverse Effects

At 4 months, DAPA reduced body weight by −2.3 ± 0.5 kg (85.2 ± 3.1 to 82.9 ± 3.2 kg), while EXE decreased body weight by 3.3 ± 1.4 kg (93.2 ± 3.7 to 89.9 ± 3.5 kg). The decrease in body weight (Δ = −7.6 kg; 92.1 ± 2.5 to 84.5 ± 2.3 kg) in patients treated with DAPA/EXE was greater than with each drug alone ($P < .01$). At 4 months there was a similar decrease in HbA1c with DAPA from 66.3 ± 2.4 to 59.1 ± 1.8 mmol/mol (8.3 ± 0.2 to 7.4 ± 0.1%) and with EXE from 63.9 ± 2.4 to 56.7 ± 2.2 mmol/mol (8.0 ± 0.2 to 7.1 ± 0.3%). DAPA + EXE combination therapy, however, produced an additive and greater reduction ($P < .01$ vs EXE alone and DAPA alone) in HbA1c from 67.1 ± 2.6 to 52.7 ± 1.5 mmol/mol (8.4 ± 0.3 to 6.6 ± 0.1%).

Among patients treated with DAPA, 6 experienced a genital mycotic infection, which responded to local therapy. Two patients had hypoglycemia upon initiation of therapy; both were on sulfonylurea. Four patients on EXE reported nausea and 1 had vomiting. In patients treated with DAPA + EXE, 5 experienced a genital mycotic infection that responded to local therapy; 3 DAPA + EXE patients had mild hypoglycemia, all on sulfonylurea; and 2 had nausea and vomiting.

Discussion

The major novel finding of this study is that impaired suppression of EGP observed during OGTT with DAPA therapy in type 2 diabetes is chronic (4 months) and persistent, despite an increase in plasma insulin and decrease in plasma glucagon concentrations. This is the first demonstration that the SGLT2i-induced stimulation of both fasting and post-OGTT glucose production is retained on a long-term basis. Our results confirm and extend previous reports (3, 4, 6, 9) by indicating that the antagonistic effect of SGLT2i on EGP does not wane over time. The mechanism(s) responsible for

SGLT2i-induced stimulation of EGP with SGLT2i have yet to be identified but cannot be attributed to changes in plasma insulin, glucagon, or glucose (6-9). However, it is reasonable to assume that the factor(s) responsible for stimulation of EGP originate within the kidney (10). Of note, the stimulatory effect of SGLT2i on EGP is not blunted in patients with type 2 diabetes who receive a transplanted kidney (11), which, by definition, is denervated, but preserve the original disease kidneys. In contrast, in polycystic kidney disease patients who undergo bilateral native nephrectomy prior to renal transplantation, the SGLT2i-induced stimulation of EGP is markedly dampened (12), demonstrating that the diseased kidney is still able to generate the signal that stimulates EGP in response to SGLT2-induced glucosuria. Stimulation of EGP by SGLT2is is rapid, occurring within 20 minutes (3, 6). This suggests that the signal responsible for the increase in EGP is related to the renal nerves, although release of blood borne factor(s) cannot be excluded.

It is currently unknown whether the increase in EGP emanates from liver, kidney, or both, although preliminary findings suggest that the increment in EGP in these conditions derives mostly from the liver and, that the contribution of the kidney is minimal (21). Changes in plasma insulin, glucagon, and glucose cannot explain the SGLT2i-induced stimulation of EGP, which occurs despite EXE-induced hyperinsulinemia and hypoglucagonemia, as well as hyperglycemia, as shown in the present study. Moreover, as previously shown (8), in this study there was an increase in baseline fasting plasma FFA in patients treated with DAPA, but not with EXE or DAPA/EXE. Considering that EGP remained elevated, whether baseline fasting FFA levels increased or decreased, changes in plasma FFA concentration also cannot explain the continued stimulation of EGP during SGLT2i-induced glycosuria. On the other hand, demonstration that suppressive effects of EXE and of an oral glucose load on EGP are dampened by coadministration of DAPA further serves to underscore the existence of this novel, potent, and sustained regulatory mechanism that links the kidney to the liver, increasing glucose release into the circulation, thereby preventing hypoglycemia. This explains why individuals with familial renal glycosuria (16) and normal glucose tolerant individuals given an SGLT2i under fasting conditions (17) do not develop hypoglycemia.

The increase in PG during OGTT was lower in patients who received DAPA + EXE vs those receiving each drug individually. This difference became progressively wider during the 1-month ($P < .05$ vs acute) and 4-month ($P < .01$ vs 1 month) studies. FPG also was significantly reduced with DAPA + EXE vs DAPA alone and EXE alone after 4 months. These novel observations indicate that there is a time-related synergistic interaction between EXE and DAPA to reduce both FPG and post-OGTT PG. The greater decline in PG is, in part, explained by the greater increase in UGE that occurred despite the much lower PG concentration during OGTT and, in part, by the increase in tissue Rd secondary to the EXE-induced stimulation of insulin secretion and amelioration of glucotoxicity (3). It should be noted that improved glycemic control (both FPG and OGTT) was observed in both the 1-month and 4-month studies with DAPA/EXE combination therapy, even in the presence of DAPA-induced rise in EGP. Despite differences in the elevation of PG concentration during the OGTT among groups in all 3 studies, when DAPA was given, either alone or in combination with EXE, the expected suppression of EGP with hyperglycemia remained impaired, which was most evident when glycemic excursion was greatest. The lower increase in MCR_G with DAPA vs EXE and the absolute decrease in MCR_G in DAPA + EXE at all times is consistent with earlier findings (4, 8). This can be explained by a shift in substrate oxidation from carbohydrate to lipid oxidation (8, 10). Lastly, the total Ra reflects 3 simultaneously ongoing processes: gastric emptying/glucose absorption, splanchnic (hepatic) glucose uptake, and EGP. During the EXE and DAPA/EXE studies (acute, 1 month, 4 months), total Ra decreased markedly compared with DAPA alone, reflecting the well-known effect of EXE to decrease gastric emptying (18) and/or possibly augment splanchnic glucose uptake (19, 20).

EXE caused a modest increase in plasma insulin concentration during OGTT during the acute study, while at 1 month and 4 months, the plasma insulin response was markedly increased ($P < .001$ vs DAPA). When DAPA was administered with EXE, the increase in plasma insulin during the acute, 1-month and 4-month studies was significantly less than with EXE alone ($P < .01$) due to the blunted rise in PG concentration secondary to marked glycosuria. Despite the rise in plasma insulin (as well as glucose) concentration during OGTT, suppression of EGP with DAPA (DAPA vs DAPA + EXE vs EXE) was impaired, emphasizing the potent link between kidney and liver to stimulate EGP. In all studies the rise in plasma C-peptide closely paralleled the increase in plasma insulin. The between group variations of plasma insulin response during OGTT can explain some of the differences in responses of glucose kinetics. For instance, the attenuated elevation in plasma insulin in the DAPA/EXE combination group had a lesser suppressive effect on EGP and lesser stimulatory effect on tissue Rd in the acute, 1-month, and 4-month studies compared with EXE alone.

As previously reported by us and others (3, 4, 7, 9, 11, 12), in the acute study the plasma glucagon concentration during OGTT increased significantly with DAPA. When EXE was administered with DAPA in acute, month 1, and month 4 studies, the plasma glucagon concentration decreased from baseline, yet DAPA-induced stimulation of EGP persisted, excluding glucagon in the stimulation of EGP. When the modest decrease in plasma glucagon is considered in context of the marked EXE-stimulated increase in plasma insulin response (ie, the INS/GCN ratio), DAPA-stimulated increase in EGP becomes even more compelling. It should be emphasized that in the current study we sought to determine only the role of insulin, glucagon, and glucose on changes in rates of endogenous glucose production, and that we did not examine any potential involvement of other factors or counter-regulatory hormone in response to SGLT2i-induced glycosuria. In agreement with previous publications (22, 23) our results have important clinical implications for the management of patients with type 2 diabetes in whom glycemic control often is suboptimal and weight loss is difficult to achieve. Initial combination therapy with DAPA + EXE produced a completely additive effect to reduce HbA1c from 67.1 ± 2.6 to 52.7 ± 1.5 mmol/mol, $\Delta = -14.4$ mmol/mol (8.4-6.6%; $\Delta = -1.8 \pm 0.2\%$) at 4 months. Equally impressive was the synergistic effect of initial DAPA/EXE combination therapy on body weight, which decreased by ~7.6 kg. Thus, even though this study was of a short duration, compared with the DURATION-8 study (22, 23) these findings provide additional evidence that SGLT-2i plus GLP1 RA represent an effective early treatment choice for type

2 diabetes patients and are consistent with the 2022 ADA/EASD standards of care, which now recommend initiating therapy in patients with type 2 diabetes with drug combination (24).

The study has some limitations. The dual-isotope technique has been used extensively to trace oral and endogenously derived glucose separately under various conditions (9, 13, 14, 19, 20). However, estimates of glucose kinetic parameters have ~10% to 15% variability (13, 14). Therefore, changes less than 15% may be difficult to observe. Moreover, using this technique we cannot discern whether the lower rates of oral glucose appearance in systemic circulation are secondary to glucose retained in stomach (eg, slowed gastric emptying) vs increased glucose uptake by the splanchnic tissues (liver and/or gastrointestinal) (19, 20). One additional limitation of this study is the lack of data in a baseline study with placebo (ie, without 1 of the study drugs), namely GLP-1 RAs and SGLT2is. Although these data were obtained and included in a separate recently published manuscript (9), in the analysis of the current studies, a true and simple comparison between metabolic effects of "taking vs not taking" a drug is not possible. Another limitation is that the study was not designed to investigate potential neuroendocrine and other humoral factors that might contribute to the stimulation of EGP in response to SGLT2i-induced glycosuria. Further investigation specifically addressing the role of the sympathetic nervous system, as well as that of circulatory mediators, is warranted. Lastly, the contribution of the kidney to the rise in EGP following SGLT2i therapy remains to be defined.

In summary, we demonstrate that glycosuria in DAPA-treated patients with type 2 diabetes causes a long-lasting elevation in endogenous glucose production despite increased plasma insulin and decreased plasma glucagon concentrations. This rise in EGP is observed with acute administration of DAPA and persists for at least 4 months. Moreover, the increased EGP in response to acute and chronic glycosuria is not affected by the ingestion of a glucose load. Based on preliminary findings, we propose that the increase in EGP emanates predominantly from the liver and identifies a novel renal–hepatic axis that is most likely neurally mediated. Further, although stimulation of insulin secretion by hyperglycemia is consistently dampened with DAPA treatment, even when DAPA is coadministered with EXE, an insulin secretagogue, improvements in fasting PG and post-OGTT glycemic excursion persist or are further enhanced as a function of time, because of sustained glycosuria and greater efficiency of tissue glucose uptake. As a result, combination therapy of EXE with DAPA produced an additive decline in HbA1c.

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Author Contributions

E.C., R.A.D. conceived the study; E.C., A.G., R.A.D. set up methods, software programs, and analyzed and interpreted the data; E.C., M.A., C.S.H., B.G., J.A., A.H.D., A.C., C.T. participated in subject recruitment, study procedures, collection of samples and materials, in laboratory analyses and some data interpretation; E.C., C.T., R.A.D. wrote the original draft; all authors reviewed, edited and approved the manuscript. E.C. and R.A.D. acquired funds; R.A.D. is the guarantor of this work.

Disclosures

R.A.D. is a member of the advisory boards of AstraZeneca, Janssen, Lexicon, Boehringer-Ingelheim-Lilly Alliance, and Novo Nordisk, a member of the speakers' bureau of Novo Nordisk, and AstraZeneca and has grant support from AstraZeneca and Janssen. E.C. has grant support from AstraZeneca. AG has received honorarium from Novo Nordisk and is consultant for Boehringer Ingelheim, Eli Lilly, Gilead, Inventive and Pfizer. C.S.H. is a member of the speakers' bureau of Novo Nordisk, and of the advisory board of Bayer. M.A., B.G., J.A., A.H.D., A.C. have no disclosures. All authors declare no conflict of interest.

Data Availability

The datasets generated during current study are available from corresponding author upon reasonable request.

Clinical Trial Information

ClinicalTrials.gov number NCT03331289 (registered November 6, 2017).

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