Glucagon Levels During Short-Term SGLT2 Inhibition Are Largely Regulated by Glucose Changes in Patients With Type 2 Diabetes

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Context: The mechanism mediating sodium glucose cotransporter-2 (SGLT2) inhibitor–associated increase in glucagon levels is unknown.

Objective: To assess short-term effects on glucagon, other hormones, and energy substrates after SGLT2 inhibition and whether such effects are secondary to glucose lowering. The impact of adding a dipeptidyl peptidase-4 inhibitor was addressed.

Design, Setting, and Patients: A phase 4, single-center, randomized, three-treatment crossover, open-label study including 15 patients with type 2 diabetes treated with metformin.

Interventions: Patients received a single-dose of dapagliflozin 10 mg accompanied by the following in randomized order: isoglycemic clamp (experiment DG); saline infusion (experiment D); or saxagliptin 5 mg plus saline infusion (experiment DS). Directly after 5-hour infusions, a 2-hour oral glucose tolerance test (OGTT) was performed.

Results: Glucose and insulin levels were stable in experiment DG and decreased in experiment D [P for difference (P_{diff}) < 0.001]. Glucagon-to-insulin ratio ($P_{diff} < 0.001$), and levels of glucagon ($P_{diff} < 0.01$), nonesterified fatty acids ($P_{diff} < 0.01$), glycerol ($P_{diff} < 0.01$), and β-OH-butyrate ($P_{diff} < 0.05$) were lower in DG vs D. In multivariate analysis, change in glucose level was the main predictor of change in glucagon level. In DS, glucagon and active GLP-1 levels were higher than in D, but glucose and insulin levels did not differ. During OGTT, glucose levels rose less and glucagon levels fell more in DS vs D.

Conclusion: The degree of glucose lowering markedly contributed to regulation of glucagon and insulin secretion and to lipid mobilization during short-term SGLT2 inhibition. (***J Clin Endocrinol Metab*** **104: 193–201, 2019)**

Type 2 diabetes (T2D) is a complex disease with genetic and lifestyle factors contributing to its pathobiology (1–4). It is characterized by hyperglycemia, typically due to impaired pancreatic β-cell function, peripheral insulin resistance, and elevated endogenous glucose production (2). Inappropriate high glucagon secretion from α cells, impaired incretin secretion and action, and increased renal glucose reabsorption also contribute to the manifestations of T2D (5–9). Some newer treatment modalities for T2D, namely sodium-glucose

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Abbreviations: AE, adverse event; aGLP-1, active glucagon-like peptide 1; AUC, area under the curve; DPP4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide 1; HbA1c, glycated hemoglobin; NEFA, nonesterified fatty acid; OGTT, oral glucose tolerance test; P_{diff}, P for difference; RA, receptor agonist; SGLT2, sodium glucose cotransporter 2; T2D, type 2 diabetes; ΔAUC, change in area under the curve.

cotransporter-2 (SGLT2) inhibitors (10–14) and glucagon-like peptide-1 (GLP-1) receptor agonists (RAs) (15, 16), respectively, have proven useful to address such perturbations. Some studies have also shown beneficial effects on cardiovascular and renal events and mortality (12–14, 17–20).

SGLT2 inhibitors block glucose reabsorption in the renal proximal tubuli, leading to urinary glucose excretion (15), which, in turn, lowers plasma glucose levels (21). The increase in urinary glucose excretion is partly, but not fully, balanced by increased endogenous glucose production (22); this has been linked to increased glucagon levels and glucagon-to-insulin ratio (11, 22, 23), as well as increased GLP-1 concentrations (22). The glucagon-to-insulin ratio is considered to largely determine endogenous glucose production rate (23, 24). The primary cause and impact of altered glucagon regulation after SGLT2 inhibition is not established.

Concerns have been raised about a potential direct effect of SGLT2 inhibitors on pancreatic α cells (25), leading to increased glucagon secretion, in turn possibly affecting glycemic control and downstream effects on energy substrate use. SGLT2 is expressed in pancreatic α cells and its inhibition triggers glucagon secretion *in vitro* (26). However, this α-cell effect is complex and appears to depend on ambient glucose levels, according to *in vitro* experiments (27, 28). If glucagon concentrations are mainly governed by prevailing plasma glucose levels during SGLT2 inhibition, the subsequently raised endogenous glucose production may largely reflect a physiological counter-regulatory response defending the established long-term glucose level.

GLP-1 RAs and dipeptidyl peptidase-4 (DPP4) inhibitors are incretin-based therapies that stimulate insulin release (29, 30), inhibit glucagon secretion (31), and lower plasma glucose levels, mainly in the postprandial state and when glucose levels are elevated (32). DPP4 inhibitors prevent enzymatic degradation of endogenous GLP-1 and improve glycemic control, with low risk for hypoglycemia (33–35). DPP4 inhibitors and GLP-1 RAs are reported to decrease levels of circulating glucagon in T2D (31), but after chronic GLP-1 RA treatment, there may instead be an increase (36, 37). DPP4 inhibitors can mitigate SGLT2 inhibitor–induced increase in postprandial glucagon secretion, and this has been demonstrated after long-term combination treatment with such drugs (38). However, it is not known if glucagon regulation is acutely affected by DPP4 inhibition in combination with SGLT2 inhibition.

In this study, we aimed to assess whether there are indirect effects of short-term SGLT2 inhibition on glucagon secretion that are mediated via glucose lowering. Likewise, we addressed whether effects of SGLT2 inhibition on other hormones and energy substrates are dependent on glucose changes. In addition, the effect of combining a DPP4 inhibitor with the SGLT2 inhibitor was investigated.

Methods

Study design

This was an investigator-initiated, exploratory, phase 4, single-dose, three-treatment crossover study assessing the acute effects of single doses of the SGLT2 inhibitor dapagliflozin on glucagon and other hormones, and on energy substrates in patients with T2D treated with metformin. Such effects were investigated during maintained and falling blood glucose levels, as well as after coadministration with the DPP4 inhibitor saxagliptin. The study was conducted between March 2016 and December 2016 at a single site, the Uppsala University Hospital in Sweden, in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines as defined by the International Conference on Harmonization, and was approved by the regional research ethics review board in Uppsala. All participants provided informed written consent. The study is registered with ClinicalTrials.gov (identifier NCT02765204) and with the European Clinical Trials Database (no. 2015-005549-30). The study comprised a screening visit (visit 0) and three intervention days (visits 1, 2, and 3), each separated by a wash-out period of 2 to 6 weeks (Fig 1).

Study population

This study included 15 male and female patients with T2D, aged 18 to 75 years, who were receiving stable metformin treatment of $\geq$ 1 month, with glycated hemoglobin (HbA1c) of 7.2% to 10% (55 to 86 mmol/mol) and body mass index of 20 to 35 kg/m^2. Patients were excluded if they had a history of type 1 diabetes, diabetic ketoacidosis, treatment with antihyperglycemic medication other than metformin or other medications known to affect glucose metabolism, or had impaired kidney function (estimated glomerular filtration rate $<$60 mL/min/1.73 m^2), signs of liver disease, any other serious ongoing illness or substance abuse. Seven patients had antihypertensive treatment and seven took statins for dyslipidemia. Patients were requested to fast overnight at least 10 hours before all the visits (moderate consumption of water was allowed up to 1 hour before visit), and to refrain from taking medication on the morning of the visits. They were instructed to continue their regular medication without changing doses or adding new medications unless advised by a physician.

Experimental procedures and treatments

All experiments started in the morning between 8:00 and 9:00 AM after an overnight fast. Patients underwent three different experiments on separate days, at least 2 weeks apart, and the order of experiments was randomly assigned according to a crossover design. The three investigational visits started with baseline measurements (time 0) taken in the fasting state and just before drug ingestion and start of infusions. The baseline sampling was directly followed by one of the three different treatments: (1) a single oral dose of dapagliflozin 10 mg plus a 5-hour isotonic saline infusion, hereafter referred to as experiment D. Patients' plasma glucose level dropped, and the change in area under the curve (ΔAUC) was $-16 \pm 1\%$ (mean $\pm$ SEM)

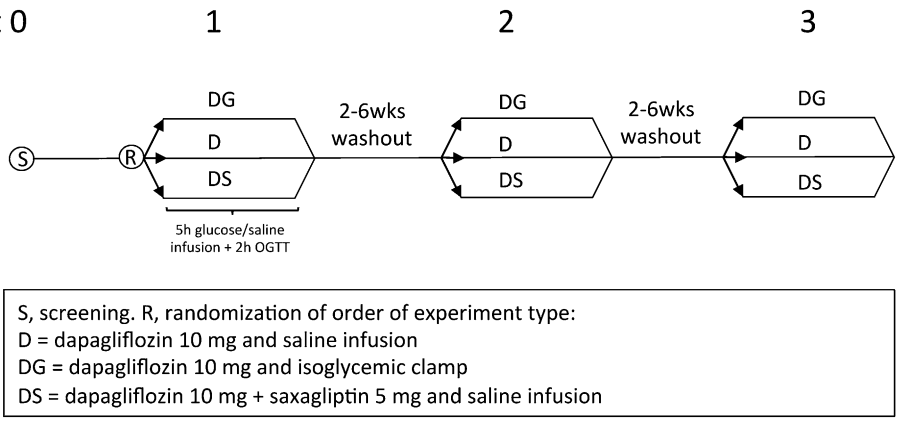

Figure 1. Study design. Each participant was scheduled for the three different 1-day experiments in randomized order.

according to measures with a handheld glucometer. (2) A single oral dose of dapagliflozin 10 mg plus isoglycemic clamp; hereafter referred to as experiment DG. Plasma glucose level was maintained at the ambient baseline level (ΔAUC 0 ± 1%) by a variable glucose infusion (10% glucose) based on bedside glucose measurements every 5 to 10 minutes. And (3) A single oral dose of dapagliflozin 10 mg plus a single oral dose of saxagliptin 5 mg plus a 5-hour isotonic saline infusion; hereafter referred to as experiment DS. Plasma glucose ΔAUC was −19 ± 2%. The single doses of dapagliflozin and saxagliptin, respectively, were expected to rapidly result in therapeutic drug concentrations in plasma throughout the experiment given their reported absorption (time to maximum concentration, <1.5 hours) and clearance rates (39, 40). For all treatments, the 5-hour infusion was immediately followed by a 2-hour oral glucose tolerance test (OGTT; 75 g).

Glucose concentrations were measured in urine collected from start to end of the infusion and OGTT periods (time 0 to 5 hours and time 5 to 7 hours, respectively), and the urine volumes were measured for these periods to calculate the amount of urinary glucose excretion (volume times glucose concentration). Levels of glucagon, insulin, active GLP-1 (aGLP-1), glucose, glycerol, and nonesterified fatty acids (NEFAs) were measured at 0, 60, 120, 180, 300, 305, 330, 360, and 420 minutes (and also at 315 minutes for hormones and glucose). β-OH-butyrate level was measured at 0, 120, 300, and 420 minutes. The amount of glucose infused was noted every 20 minutes during the 5-hour glucose infusion for calculation of glucose infusion rate.

All measurements were performed using certified routine methods at the central laboratory of the Department of Clinical Chemistry at the Uppsala Academic Hospital or at the in-house diabetes research laboratory. Glucose monitoring to adjust the glucose infusion during isoglycemic clamps was performed bedside with a handheld device (Contour XT; Bayer Healthcare, Leverkusen, Germany). β-OH-butyrate level was also measured bedside (FreeStyle Precision Neo; Abbott Diabetes Care, Witney, United Kingdom). Samples for glucagon and aGLP-1 measurements were collected in tubes containing protease inhibitors, including sitagliptin (P800; Becton Dickinson, Franklin Lakes, NJ). These and other samples that were not immediately analyzed were centrifuged upon collection, aliquoted, and frozen at −80°C. Active GLP-1 was assessed with an electrochemiluminescence kit (MesoScale Discovery, Rockville, MD; sensitivity, 0.12 pg/mL), glucagon with ELISA (Mercodia AB, Uppsala, Sweden; sensitivity: 1 pmol/L), glycerol with free glycerol reagent (Sigma-Aldrich, St. Louis, MO) and NEFA with fluorometric assay kit (Cayman Chemical, Ann Arbor, MI). All samples were centrifuged immediately. To reduce intrasubject variability, all samples from each participant were assayed together.

Outcome measures

In this exploratory study, the primary objective was to evaluate whether a single dose of dapagliflozin exerted an indirect effect on human pancreatic α cells via glucose changes, in turn affecting glucagon secretion. The primary end point was the relative change from baseline in glucagon concentration during isoglycemia vs spontaneous glucose lowering, after SGLT2 inhibition. Other objectives were to assess whether the acute effects of SGLT2 inhibition on insulin, aGLP-1, and energy substrates depend on glucose levels. Experiment DS was conducted to specifically investigate whether concomitant DPP4 inhibition acutely modifies levels of glucagon and other hormones, in comparison with SGLT2 inhibition alone (experiment DS vs experiment D). The investigations were performed both in the fasting condition and after OGTT. Safety was assessed by reports of adverse events (AEs) and serious AEs.

Statistical methods

The study had 90% power to detect a 15% difference in the primary end point between the two conditions in the primary comparison. Based on previous work, this difference was judged to be clinically relevant and calculated to be 3 pmol/L (baseline level of about 20 pmol/L; SD, 3 pmol/L) (11). Thus, the sample size required for analysis of the primary end point was 12 evaluable patients. A maximum of three patients who did not complete the study (premature withdrawals) was expected. Thus, 15 patients were enrolled and randomly assigned to one of the six possible treatment orders.

In this crossover study, each patient served as his or her own control. All analyses were done on the full analysis set, including all 15 patients who were assigned randomized treatment orders and took at least one dose of the study medication. The safety analysis set included all patients receiving any study drug; this also constituted the full analysis set.

Data are presented as mean ± SD or SEM, as indicated, and have been log-transformed when not normally distributed

according to Shapiro-Wilks test (nonnormal, <0.05). The primary end point was analyzed using a paired Student *t* test, with two-sided α set at 0.05. No adjustments were made for multiple comparisons and all other analyses were performed in an exploratory manner. Effects on hormone and substrate levels are expressed as relative ΔAUC (% change) from the baseline level at start of the 5-hour infusion or the start of the 2-hour OGTT, respectively. AUCs for these two separate periods were derived using the trapezoidal rule. ΔAUC was used in two main comparisons between experiment types, DG or DS, vs D.

To quantify the effects of individual variables on the changes in hormones and energy substrates, multivariate regression analyses using the forward stepwise method and including the variables sex, subject, experiment type, and plasma glucose and insulin or glucagon levels were conducted on pooled data from experiments DG and D. All data were analyzed with SPSS, version 24 (IBM).

Results

Overall, 29 patients were screened and 15 patients (12 men, 3 women) fulfilling inclusion criteria entered the study and completed at least one experiment. At baseline, the patients' mean HbA1c was 56.5 mmol/mol (7.3%), mean body mass index was 27.1 kg/m^2, and time since the diagnosis of T2D was 7.4 years (Table 1). Seven patients were treated for hyperlipidemia and seven for hypertension. In total, three patients discontinued the study prematurely; of these, one withdrew consent, one withdrew because of AEs, and one withdrew because of venous catheter failure. Twelve patients completed all three visits and two more (*i.e.* 14 in total), completed at least two experiments. The number of patients analyzed was 13 for the primary comparison of dapagliflozin with glucose clamp or saline infusion (experiment DG vs experiment D) and 12 for dapagliflozin with vs without the addition of the DPP4 inhibitor saxagliptin (experiment DS vs experiment D).

Table 1. Clinical Characteristics of Study Participants

Characteristic	Mean ± SD (N = 15)
Sex, male/female, no.	12/3
Age, y	67 ± 6
T2D duration, y	7.4 ± 4.2
BMI, kg/m^2	27.1 ± 2.9
Waist-to-hip ratio	1.0 ± 0.1
HbA1c, mmol/mol /%	56.5 ± 5.8 /7.3 ± 0.8
Fasting plasma glucose, mmol/L	9.6 ± 1.1
Metformin dose, mg/d	1933 ± 594
eGFR MDRD, mL/min/1.73m^2	97 ± 18
Systolic blood pressure, mm Hg	142 ± 11

Data are given as no. or mean ± SD.

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease formula.

Fasting state

Dapagliflozin treatment with or without isoglycemic clamp (experiments DG and D)

During the 5-hour infusion periods (time 0 to 300 minutes), central laboratory measurements confirmed that plasma glucose levels remained close to baseline levels during experiment DG (ΔAUC for 0 to 5 hours, 3% increase), but decreased steadily during experiment D [ΔAUC for 0 to 5 hours, −17%; *P* for difference (P_{diff}) between experiments DG and D < 0.001; Fig. 2A]. Glucagon levels decreased significantly more from baseline during experiment DG than experiment D (−20% vs −9%; $P < 0.001$ and < 0.01 respectively; $P_{diff} < 0.01$; Fig. 2B).

Insulin levels were not significantly changed during experiment DG but decreased during experiment D (−31%; $P < 0.001$; $P_{diff} < 0.001$; Fig. 2C). Levels of C peptide increased during experiment DG (16%) and decreased during experiment D (−11%); C-peptide levels were significantly different between the experiment types ($P_{diff} < 0.001$). The glucagon-to-insulin ratio decreased by 25% ($P < 0.001$) during experiment DG and increased by 37% ($P < 0.001$) during experiment D ($P_{diff} <$ 0.001; Fig. 2D). Levels of aGLP-1 decreased similarly from baseline during experiments DG ($P < 0.05$) and D ($P < 0.001$). Urinary glucose excretion was numerically, but not significantly, higher in experiment DG (17.0 g ± 2.6 g) than in experiment D (13.2 g ± 1.1 g) during the 5-h infusion periods.

Glycerol and NEFA decreased during experiment DG (Fig. 2E and 2F) and increased during experiment D ($P_{diff} < 0.01$). β-OH-butyrate level did not increase in experiment DG but increased significantly in experiment D (Fig. 2G).

Associations between glucose measures and hormones and substrates

In a multiple regression analysis including plasma glucose change, subject and type of experiment, change in plasma glucose was the only significant predictor of change in glucagon during experiments DG and D ($P <$ 0.01; $r^2 = 0.23$ for model). Using the same approach, only type of experiment (DG vs D) predicted change in insulin, glucagon-to-insulin ratio, and changes in NEFA and β-OH-butyrate levels. This may suggest that other mediators together with glucose were of importance for changes in these variables. Moreover, urinary glucose excretion and change in glucagon level did not correlate significantly with each other in experiments DG or D, and change in glucagon was not correlated with glucose infusion rate in experiment DG (data not shown).

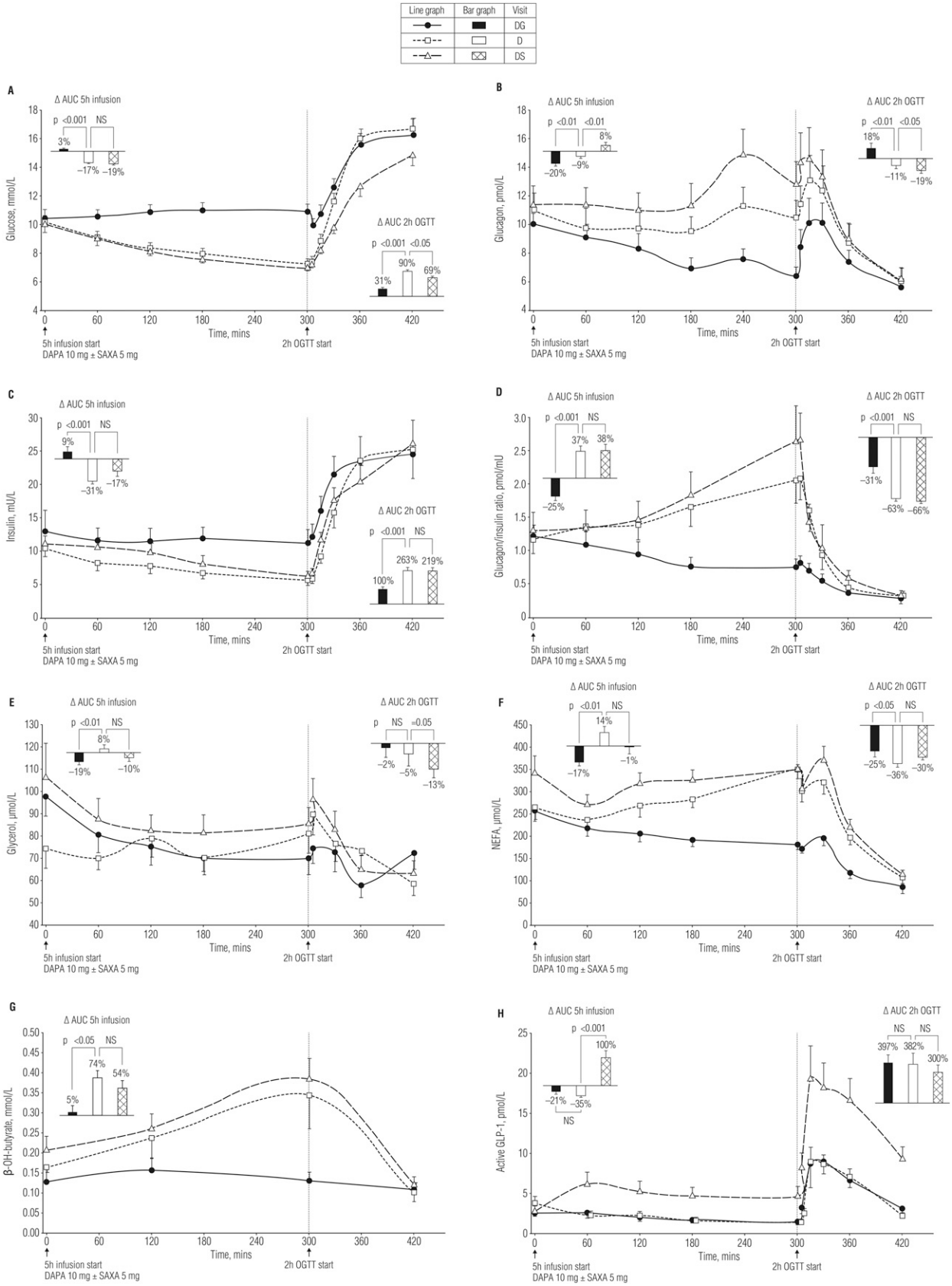

Figure 2. Change during glucose or saline infusion and during OGTT in the levels of: (A) glucose; (B) glucagon; (C) insulin; (D) glucagon-to-insulin ratio; (E) glycerol; (F) NEFA; (G) β-OH butyrate; and (H) aGLP-1. Data are given as mean ± SEM. A total of 14 patients completed the dapagliflozin (DAPA) plus isoglycemic clamp experiment, 14 completed the DAPA plus saline experiment, and 13 completed the dapagliflozin plus saxagliptin (SAXA) experiment. ΔAUC is reported for time 0 (baseline, just before drug ingestion and start of infusions) to 300 min; or from time 300 to 420 min (OGTT). NS, nonsignificant.

Dapagliflozin with or without saxagliptin (experiments DS and D)

Active GLP-1 and glucagon levels increased significantly with saxagliptin added in experiment DS vs experiment D. The glucagon-to-insulin ratio and levels of insulin, glucose, glycerol, NEFA, and β-OH-butyrate did not differ significantly with the addition of saxagliptin.

OGTT

OGTT results are shown in Fig. 2A–2H from time 300 to 420 minutes. After the isoglycemic clamp in experiment DG, plasma glucose level started higher and increased less during OGTT when compared with experiment D. In experiment DS, with saxagliptin added, glucose started at similar levels but increased less than in experiment D. Glucagon levels were overall lower but rose in experiment DG as compared with experiments D and DS. In all experiments, insulin, C peptide, and aGLP-1 levels increased, whereas the glucagon-to-insulin ratio and glycerol and NEFA levels decreased. Urinary glucose excretion during OGTT was not significantly different between experiments (data not shown).

Safety

In general, the study treatments were well tolerated; 19 AEs were reported in six patients. No new and unexpected AEs were observed. The most commonly reported events were headache (six events in three patients) and tiredness (five events in three patients). Fifteen events were mild and two (ureteral stone and urticaria) were moderate in intensity. One event, deep vein thrombosis of the arm, was deemed severe in intensity and resulted in the patient withdrawing from the study. The moderate and severe AEs required medication but none required hospitalization. Only one event (dizziness) was deemed as possibly related to the study drug. No deaths or serious AEs were reported during the study.

Discussion

The primary objective of this study was to investigate whether acute SGLT2 inhibition in patients with T2D affected glucagon secretion differently when glucose levels were kept stable vs when they were allowed to fall. We found no increase in glucagon levels during 5 hours after dapagliflozin administration, and glucagon levels fell significantly more during isoglycemic clamp compared with saline infusion. The glucagon-to-insulin ratio decreased with glucose infusion but increased with saline infusion.

Dapagliflozin with or without isoglycemic clamp

In the fasting state with glucose infusion, the plasma glucose level was stable throughout the 5-hour isoglycemic clamp experiment. In this setting, in total, ~45 to 50 g of glucose was administered (*i.e.*, considerably more than the concomitant urinary glucose excretion of ~17 g).

Conversely, during saline infusion, an overall decrease in plasma glucose AUC was seen and the final glucose level dropped by about 2.8 mmol/L by 5 hours after dapagliflozin administration. This is similar to previous findings (5) and is a consequence of the ~13 g of glucose excretion during the same period in our patients.

Upon dapagliflozin dosing, there was a significant difference in glucagon levels, and, presumably, secretion, with stable vs decreasing plasma glucose levels. No increase in glucagon levels was seen during the isoglycemic clamp, which suggests that maintaining moderate hyperglycemia leads to suppressed glucagon secretion even in the presence of an SGLT2 inhibitor. This may partly account for the difference in glucose quantity required to maintain isoglycemia and glucose quantity lost via urinary excretion after SGLT2 inhibition. Thus, reduced glucagon levels and glucagon-to-insulin ratio would also be expected to reduce endogenous glucose production and potentially increase glucose uptake and storage as glycogen in liver and muscle and as triglycerides in adipose tissue. The absence of glucagon increase in this study is consistent with previous findings on α-cell physiology. Minimal α-cell stimulation is seen at plasma glucose levels of 6 to 13 mmol/L (27), and the glucose levels in our patients were not below this range even when allowed to drop. Different studies have reported varying glucagon responses to acute SGLT2 inhibitor dosing. One study reported a glucagon increase (11), whereas other studies have reported glucagon trajectories apparently similar to our findings (22, 41).

The reasons for the different glucagon trajectories reported in different studies are not clear. However, there is some evidence that varying baseline glucose levels may play a role (27), and poor glycemic control in T2D has been associated with higher glucagon levels (42). The different glucagon assays with varying specificities that were used potentially also could contribute to different results (37). The glucagon concentrations measured in our studies were somewhat lower than what was found in similar studies on SGLT2 inhibitor administration in T2D (11, 22). This may be explained by the high sensitivity and specificity of the Mercodia ELISA for glucagon used in this study (37).

As expected, insulin and C-peptide levels followed the direction of plasma glucose levels during the experiments, and they tended to increase from baseline during glucose infusion (isoglycemia) and decreased together with the plasma glucose levels during saline infusion. The glucagon-to-insulin ratio fell from baseline during isoglycemic clamp (experiment DG) and this was different

from the setting of falling plasma glucose (experiment D) when the ratio gradually increased. Although it is not possible to distinguish between direct and indirect (*e.g.*, via insulin) effects of glucose changes on glucagon secretion, plasma glucose was the controlled factor by experimental design. Furthermore, multivariate analyses suggest that glucose levels *per se* are more important than the (secondary) changes in insulin. Overall, the α and β cells of the pancreatic islets appeared to be more responsive to changes in plasma glucose levels than to SGLT2 inhibition *per se*. Active GLP-1 levels were significantly decreased after dapagliflozin administration, possibly related to prolonged fasting, but there were no significant differences between the stable vs falling glucose settings.

Levels of NEFA, glycerol, and ketones were lower during the glucose vs saline infusion, probably reflecting relatively more glucose oxidation when some degree of hyperglycemia was maintained. Conversely, there was probably a shift toward lipid mobilization via lipolysis and lipid oxidation after SGLT2 inhibition with saline infusion, with accompanying falling glucose.

This is probably partly explained by the differences in insulin levels and glucagon-to-insulin ratio. These findings are in agreement with previous work (43) and support that when glucose lowering occurs after SGLT2 inhibition, energy metabolism in various tissues partly shifts from glucose toward fatty acid oxidation.

Dapagliflozin with or without saxagliptin

The addition of saxagliptin together with dapagliflozin rapidly increased the levels of aGLP-1 during the fasting condition. This is in line with the known pharmacokinetics and pharmacodynamics of saxagliptin. However, surprisingly, the addition also resulted in increased glucagon levels. There were no clear effects on levels of insulin or C peptide, or on energy substrates in these short-term experiments. The findings for glucagon are apparently different than those of clinical trials of several weeks' duration (44) or with a single dose of GLP-1 RA added to SGLT2 inhibitor (45). This may be explained by our single-dose setting addressing only acute effects, which probably differ from effects after chronic dosing and possibly by DPP4 inhibitors affecting other peptides, such as GIP, in addition to increasing aGLP-1 levels. Furthermore, varying sensitivity and specificity between glucagon assays may contribute to these differences (37).

OGTT

During the OGTT, there was a smaller increase in plasma glucose levels after glucose vs saline infusion. This could potentially be explained by a lower glucagon-to-insulin ratio at the start of the OGTT, but the different starting glucose levels could also play a key role. Overall, differences in glucagon and insulin profiles during OGTT reflect differences in levels at the start of the OGTT. However, similar levels of glucagon and insulin were observed at the end of the 2-hour period.

As expected, increased aGLP-1 levels during the OGTT were accompanied by a lower glucose excursion. However, insulin and C-peptide levels were not significantly affected by saxagliptin additions and neither were energy substrates. Overall, glucagon levels decreased more during the 2-hour OGTT when saxagliptin was added. Taken together, the data on glucose and aGLP-1 levels during OGTT are compatible with expected actions of a DPP4 inhibitor, whereas the lack of insulin response appears surprising. Possibly, the single-dose setting could explain differences to findings in trials with repeated dosing.

Limitations

There are several limitations to this study. First, the absolute effect of the SGLT2 inhibitor on glucagon levels could not be quantified, towing to the absence of placebo treatment, which was not feasible because the study already included three demanding investigational days. However, no major change during a 5-hour extension of an overnight fast (at least 10 hours) is to be expected (11). In particular, it would not be expected to result in a greater glucagon lowering than what was found here after dapagliflozin dosing, and thus our data suggest that there was no acute effect of SGLT2 inhibition *per se* to raise glucagon. Second, OGTT was started at different plasma glucose levels in the different experimental settings; therefore, those results need to be interpreted with caution. Third, although this study focuses on the glucose modulation of acute effects of SGLT2 inhibition, findings upon chronic SGLT2 inhibition may differ substantially (44). Fourth, long-term glycemic control may affect glucagon responses in T2D (42). The patients in our study, on average, had an acceptable glycemic control, which might be an explanation for the lack of glucagon increase after SGLT2 inhibitor reported in patients with higher HbA1c levels (11). Fifth, all recruited patients were receiving metformin (albeit not on the days of investigations until completed) and this could potentially interfere with the responses to the experimental conditions. Nonetheless, metformin doses were not changed during the study and end point comparisons were made within subjects; therefore, differences found should be due to the respective experimental conditions. Last, we cannot quantify the indirect glucose-mediated vs any direct α-cell effects of SGLT2 inhibitor on glucagon secretion, although our results suggest that the former contributes substantially.

Our findings indicate that glucagon levels and glucagon-to-insulin ratio during acute SGLT2 inhibition are largely mediated via glycemic changes, and the direct effect of SGLT2 inhibition on pancreatic α cells probably has limited short-term impact in patients with T2D. In the fasting condition, no increase in glucagon level was seen after SGLT2 inhibition. Decreases in plasma glucose levels (via urinary excretion) can markedly contribute to effects of SGLT2 inhibition on glucagon and insulin secretion from islet cells and to the mobilization of fatty acids as energy substrates. A single dose of a DPP4 inhibitor added to an SGLT2 inhibitor did not produce the typical insulin and glucagon changes seen with long-term treatment and, in fact, glucagon levels were increased.

In summary, these data suggest that for glucagon regulation after acute SGLT2 inhibition, direct islet-cell effects are of less importance than indirect effects exerted by changes in glucose levels. Additional studies are warranted to address underlying mechanisms as well as the possible changes of such effects over time during long-term SGLT2 inhibition. Taken together, these results may suggest that glucagon changes during SGLT2 inhibitor treatment largely occur as a response to glucose lowering rather than via direct drug effects on the pancreatic islets.

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