



Metabolic Effects of an SGLT2 Inhibitor (Dapagliflozin) During a Period of Acute Insulin Withdrawal and Development of Ketoacidosis in People With Type 1 Diabetes

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OBJECTIVE

To determine the effect of the sodium–glucose cotransporter 2 inhibitor dapagliflozin on glucose flux, lipolysis, and ketone body concentrations during insulin withdrawal in people with type 1 diabetes.

RESEARCH DESIGN AND METHODS

A double-blind, placebo-controlled crossover study with a 4-week washout period was performed in 12 people with type 1 diabetes using insulin pump therapy. Participants received dapagliflozin or placebo in random order for 7 days. Stable isotopes were infused to measure the glucose R_a , R_d , and lipolysis. At isotopic steady state, insulin was withdrawn, and the study was terminated after 600 min or earlier if blood glucose reached 18 mmol/L, bicarbonate <15 mmol/L, venous pH <7.35, or capillary ketones >5.0 mmol/L.

RESULTS

At baseline, glucose R_a was significantly higher for the dapagliflozin group than the placebo group. Following insulin withdrawal, plasma glucose concentrations at the end point were significantly lower with dapagliflozin than placebo and glucose R_d area under the curve (AUC)_{0–180 min} and β -hydroxybutyrate (BOHB) AUC_{0–180 min} were significantly higher. There was a small but significantly higher glycerol R_a (measure of lipolysis) AUC_{0–180 min} with dapagliflozin. Nonesterified fatty acid concentrations were not different between treatments. When divided by BMI >27 and <27 kg/m², basal glucose R_a , BOHB, and glycerol R_a AUC_{0–180 min} were significantly higher in the low-BMI group with dapagliflozin treatment versus the low-BMI group with placebo.

CONCLUSIONS

During insulin withdrawal, the increase in BOHB with dapagliflozin may be partially due to increased lipolysis. However, reduced renal excretion, reduced BOHB uptake by peripheral tissues, or a metabolic switch to increased ketogenesis within the liver may also play a role.

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Sodium–glucose cotransporter 2 (SGLT2) inhibitors are selective and reversible inhibitors of renal SGLT2, the major transporter responsible for renal glucose reabsorption. They lower plasma glucose by reducing renal glucose reabsorption and enhancing urinary glucose excretion. In type 2 diabetes, this results in a compensatory increase in endogenous glucose production, lower tissue glucose uptake, and higher glucagon levels (1).

It has been postulated that the SGLT2 inhibitor class may have beneficial effects in the management of type 1 diabetes. Phase 2 and 3 clinical trial studies have shown encouraging results, with lower HbA_{1c} levels and reduced weight and insulin requirements (2–7). However, significant safety issues have been highlighted in clinical trials, with increased rates of ketoacidosis, although rates differed between trials. In Dapagliflozin Evaluation in Patients With Inadequately Controlled Type 1 Diabetes (DEPICT-1), diabetic ketoacidosis (DKA) occurred in 1% and 2% of the dapagliflozin 5 mg and 10 mg groups, respectively (2). Pooled data from the 26-week Empagliflozin as Adjunctive to Insulin Therapy Over 52 Weeks in Patients With Type 1 Diabetes Mellitus (EASE)-2 and EASE-3 studies showed DKA occurred in 0.8%, 3.3%, and 4.3% of the empagliflozin 2.5 mg, 10 mg, and 25 mg arms, respectively, and 1.2% in the placebo arm (4). In the Efficacy, Safety, and Tolerability Study of Sotagliflozin as Adjunct Therapy in Adult Patients With Type 1 Diabetes Mellitus Who Have Inadequate Glycemic Control With Insulin Therapy (Tandem1), euglycemic ketoacidosis occurred in 3.4% and 4.2% of the sotagliflozin 200 mg and 400 mg groups and 0.4% in the placebo group over 24 weeks (6). Finally, in an 18-week randomized study with canagliflozin, ketone-related adverse events occurred in 5.1% of the 100 mg arm and 9.5% of the 300 mg arm (7).

The U.S. Food and Drug Administration has not approved SGLT2 inhibitors for use as an adjunct therapy in type 1 diabetes and have warned about the risk of euglycemic DKA. A number of triggers for ketoacidosis were identified. These included reduced calorie intake, lower insulin doses, and illness. It is also important to note that very few participants experienced a state of illness or intercurrent stress during the reported clinical

trials. A recent study estimated real world experience rates of DKA with off-label use of SGLT2 inhibitors in patients with type 1 diabetes to be higher than expected based on the sotagliflozin clinical trials (8). However, in March 2019, dapagliflozin was approved in Europe for people with type 1 diabetes as an adjunct to insulin in people with a BMI >27 kg/m² (9) and received an evidence-based recommendation by the National Institute for Clinical Excellence in August 2019 (10).

The incidence of DKA is between 0 and 56 cases per 1,000 person-years among people with type 1 diabetes (11); the pathogenesis of DKA is well-known. In the absence of adequate direct insulin action in the liver, the excess glucose production is so great that an increase in glucose-dependent peripheral glucose uptake and associated glycosuria fail to limit progressive hyperglycemia. Lipolysis is increased due to lack of insulin's action on hormone-sensitive lipase resulting in increased release of nonesterified fatty acids (NEFA) and glycerol from adipose triglyceride stores (12). In the liver, due to the diversion of oxaloacetate toward gluconeogenesis, there is a reduced capacity of the Krebs cycle to oxidize acetyl-CoA derived from the β -oxidation of NEFA. Consequently, there is an increased flux of acetyl-CoA toward ketogenesis giving rise to increased acetoacetate, β -hydroxybutyrate (BOHB), and acetone (13). Hence, in insulin deficiency, there are two parallel metabolic processes: glucose overproduction and ketone overproduction.

The potentially life-threatening state of euglycemic DKA is unusual and was first described in 1973 (14). A speculative mechanism for its development with SGLT2 inhibitors is that the insulin-independent removal of glucose from the body enables glycemic control concurrent with either an absolute or a relative deficiency of insulin. In addition, ketoacidosis may be driven by an increase in counterregulatory hormones, i.e., glucagon, cortisol, or growth hormone. Using stable isotope techniques, this study explored these potential mechanisms by studying the effect of the SGLT2 inhibitor dapagliflozin on glucose flux, lipolysis, and ketone body concentration during hyperglycemia in people with absolute endogenous insulin deficiency.

RESEARCH DESIGN AND METHODS

A single-center, investigator-led, double-blind, placebo-controlled crossover study with a 4-week washout period was performed in patients with type 1 diabetes using insulin pump therapy. Ethics approval was granted from the National Research Ethics Service committee, South Central–Berkshire B. The clinical trial was registered with the European Clinical Trials Database (EudraCT) under the number 2015-002094-38.

Participants with type 1 diabetes for >12 months were recruited between February 2018 and October 2018 with use of the diabetes insulin pump database at the Royal Surrey County National Health Service Trust. Type 1 diabetes was established by clinical presentation, treatment response, and C-peptide level. Exclusion criteria included proliferative retinopathy requiring acute treatment within the last 3 months, moderate-to-severe renal impairment (creatinine clearance <60 mL/min or estimated glomerular filtration rate <60 mL/min/1.73 m²), severe hepatic impairment, New York Heart Association class III–IV cardiac failure, uncontrolled cardiac arrhythmia, uncontrolled hypertension, mental incapacity, pregnancy, or breastfeeding. Those with child-bearing potential not taking adequate contraception precautions and those with suspected allergy to trial products were also excluded.

Design

Participants received dapagliflozin (10 mg daily) or placebo in random order for 7 days. They were made aware of potential changes in glycemic control and were asked to record trial medication administration, any concomitant medication (to include insulin), hypoglycemia frequency (capillary glucose level <4 mmol/L), fasting ketone levels, and any adverse events.

On day 7, participants attended for a metabolic study. They were asked not to consume food and to drink only water from 2200 h the day before. They were also asked not to undertake any strenuous exercise or consume alcohol for 24 h before the study day. All participants were using continuous subcutaneous insulin therapy and disconnected their insulin pumps at 0600 h on the morning of a metabolic study. They were transferred to a soluble variable insulin infusion to maintain a whole-blood concentration of

glucose at 5 mmol/L. [6,6-²H₂]glucose and [1,1,2,3,3-⁵H₂]glycerol were infused from -120 min to the study end point to measure glucose R_a and R_d and lipolysis, respectively (Cambridge isotopes; CK Gas Products, Ibstock, U.K.).

At 0 min, when isotopic steady state had been achieved, insulin was withdrawn, participants were given a single dose of study medication, and blood glucose was allowed to increase.

Blood samples were taken to measure plasma glucose and glycerol enrichment and concentration and concentrations of NEFA and plasma BOHB every 20 min until 180 min and then at 30-min intervals. Interval urine collection was also taken, and blood samples for counter-regulatory hormones were taken to measure plasma glucagon and serum insulin, growth hormone, and cortisol concentrations at 120-min intervals. Urine samples were collected over 2-h intervals for measurement of glucose and spot urinary ketones.

Rescue and Study Termination

At 600 min (10 h) or in the event of blood glucose of 18 mmol/L, bicarbonate <15 mmol/L, or venous pH <7.35 or point-of-care capillary ketone level of >5.0 mmol/L, the metabolic study was terminated and participants commenced on rescue intravenous insulin infusion and 5% dextrose until blood glucose levels stabilized.

Plasma Measurements

On the study day, plasma glucose concentration was measured using a glucose oxidase technique on a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Whole-blood samples were immediately centrifuged and aliquots of plasma stored at -80°C to be analyzed at a later date in a laboratory setting.

Plasma glucose concentrations were measured with a Roche Cobas Mira analyzer using the ABX Pentra glucose kit (HORIBA ABX, Northampton, U.K.) and plasma glycerol and BOHB concentrations using Randox kits (Glycerol and Ranbut; Randox Laboratories, Co. Antrim, U.K.). Plasma NEFA concentrations were measured using an enzymatic kit from Wako Chemicals (Neuss, Germany).

Insulin and glucagon were measured using radioimmunoassays purchased from Merck Millipore, Merck Chemicals

(Nottingham, U.K.). Serum cortisol concentration were measured using an in-house radioimmunoassay. Serum growth hormone concentrations were measured using an immunoradiometric kit purchased from DRG Instruments, supplied by IDS, Tyne and Wear, U.K. The isotopic enrichment of plasma glucose was determined as the trimethylsilyl-*O*-methyloxime derivative (15) using gas chromatography-mass spectrometry (model 5975C, inert XL EI/CI MSD; Agilent Technologies, Wokingham, U.K.). The isotopic enrichment of plasma glycerol was determined as the *tert*-butyl trimethylsilyl glycerol derivative (16) using a gas chromatography-mass spectrometry model 5975 network MSD (Agilent Technologies).

Glucose R_a, R_d, and glycerol production were calculated using the Steele non-steady state equations modified for stable isotopes (17).

Statistical Analysis

Statistical analysis was performed using R, version 3.5.1, and SAS, version 9.4 or above. All hypothesis tests were two sided and evaluated at a significance level of 5%.

The primary end point was glucose concentration as measured at 600 min or at the time of glycemic rescue—whichever occurred first. The study was powered for 12 subjects. In a recent study where insulin was withdrawn in people with type 1 diabetes, the SD for plasma glucose concentration was 20% (18). With 12 subjects, studied with and without the SGLT2 inhibitor, a difference in plasma glucose of 24% can be detected with 80% power and two-sided significance level of 5%.

Final glucose concentration was statistically analyzed as the response variable in a general linear mixed model (using the PROC MIXED procedure in SAS software), with treatment, period, and treatment-by-period interaction as fixed effects and the baseline glucose concentration as a covariate. The participant was a random effect in the model.

The secondary end points were statistically evaluated as response variables using a general linear mixed model with treatment, period, time, treatment-by-period, and treatment-by-time interactions as fixed effects, baseline measurement as a covariate, participant as a random effect, and time as a repeated measure, with SP(POW) variance-covariance structure.

Denominator degrees of freedom were adjusted using the Kenward-Roger approximations. All other response variables measured or derived once per participant in each period were analyzed as per the primary end point.

For assessment of the impact of participant BMI level on study conclusions, the participants were classified as “high BMI” (BMI >27 kg/m², *N* = 5) or “low BMI” (BMI <27 kg/m², *N* = 7). The data for which a single value per participant per period was analyzed were then re-analyzed as described above with the modification that the model independent variables included, additionally, the BMI classification and the interaction of the BMI classification with treatment.

The data for which several time point values per participant per period were analyzed were then reanalyzed as described above with the modification that the model independent variables included, additionally, the BMI classification, the interaction of the BMI classification with treatment, and the interaction of the BMI classification, treatment, and time point. For each separate time point, the same additional estimates of effect and *P* value for BMI and treatment combined, as immediately above, were reported.

Area under the curves were calculated for study points 0–180 min with and without correction for the baseline. In the figures, the mean values at each time point only include data where there is a measurement from both arms of the study at that time point. We have called these paired measurements.

RESULTS

Twelve people (4 male and 8 female) with type 1 diabetes, with mean ± SEM duration of diabetes 23.3 ± 4.1 years (age 40.7 ± 3.9 years, BMI 26.8 ± 1.4 kg/m², HbA_{1c} 59.9 ± 2.3 mmol/mol), completed the study. All participants had C-peptide <0.2 nmol/L, apart from one participant who had a C-peptide level of 0.314 nmol/L and 0.352 nmol/L in the dapagliflozin versus placebo arm, respectively, with duration of type 1 diabetes of 17 years.

During the 7-day treatment period, there was no significant difference in insulin dose between dapagliflozin versus placebo (mean ± SEM 0.056 ± 0.007 units/kg vs. 0.058 ± 0.008 units/kg, respectively). Two participants reduced their basal setting due to hypoglycemia. Other participants did not change their

insulin dose but encountered more episodes of hypoglycemia overnight, which were remedied by taking GlucoGel (formerly known as HypoStop gel).

The duration of each metabolic study prior to termination or rescue varied from 180 to 600 min. All subjects completed 180 min of each metabolic study. The average time for each metabolic study was not different between dapagliflozin (mean \pm SEM 418 \pm 44 min) and placebo (448 \pm 38 min).

Glucose Concentration

At 0 min, glucose concentration was not different between treatments (Fig. 1). Following insulin withdrawal, plasma glucose concentration increased in both groups, but at the end of the study (600 min or time of rescue) the mean \pm

SEM glucose concentration was 8.5 \pm 0.7 mmol/L with dapagliflozin treatment and 14.3 \pm 1.1 mmol/L with placebo ($P = 0.0005$) (Fig. 1). Urinary glucose excretion between 0 and 120 min ($n = 6$) was 5.10 \pm 0.80 μ mol/kg/min with dapagliflozin and 0.029 \pm 0.01 μ mol/kg/min with placebo ($P = 0.003$).

Glucose Metabolism

At baseline (0 min), when isotopic enrichment and glucose concentration were in a steady state, glucose R_a was significantly higher with dapagliflozin compared with placebo ($P = 0.0088$) (Fig. 1 and Table 1). At this time point, glucose R_d is equal to glucose R_a . During insulin withdrawal, glucose R_a increased, peaking at 90 min, and then declined, with no difference in AUC between

treatments. AUC_{0–180 min} for glucose R_d and metabolic clearance rate (MCR) were higher with dapagliflozin compared with placebo ($P = 0.0041$, $P < 0.0001$).

BOHB, NEFA, and Glycerol Metabolism Baseline

There was no difference in baseline glycerol R_a (a measure of lipolysis) between treatments (Fig. 2 and Table 1). Baseline BOHB was higher with dapagliflozin (mean \pm SEM 0.39 \pm 0.11 vs. 0.16 \pm 0.05 mmol/L, $P = 0.044$), and NEFA concentration was higher (0.61 \pm 0.09 vs. 0.47 \pm 0.07 mmol/L), although this was borderline significant ($P = 0.054$). There was a significant positive relationship between NEFA and BOHB with both treatments (Supplementary Fig. 3). Base excess concentration and venous bicarbonate

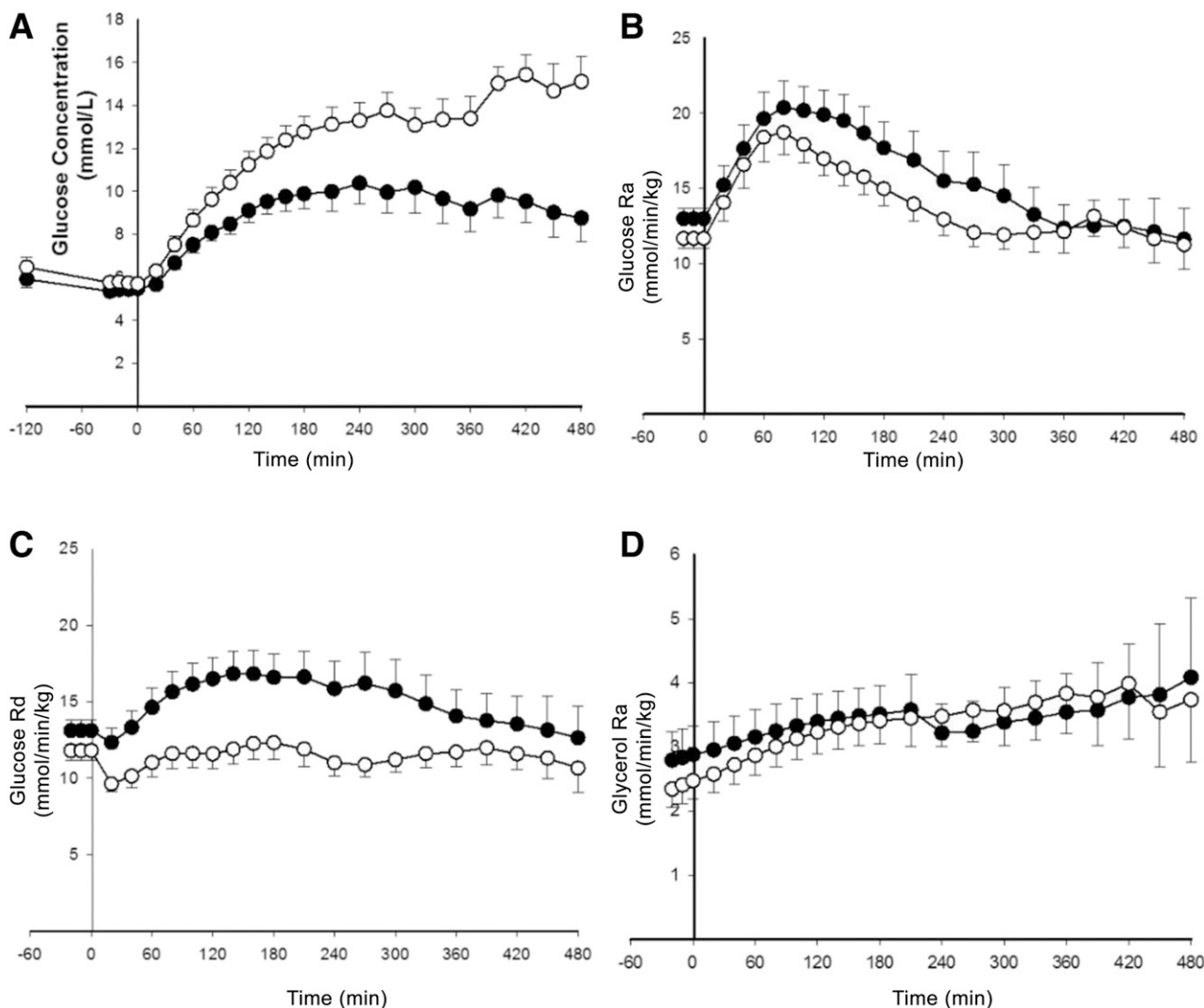


Figure 1—Paired plasma glucose concentration (A), glucose R_a (B), glucose R_d (C), and glycerol R_a (D). All subjects completed 180 min of each metabolic study ($n = 12$). By 480 min, $n = 5$. \circ , placebo; \bullet , dapagliflozin.

Table 1—Baseline AUC and incremental AUC from 0 to 180 min for glucose, glycerol, and BOHB metabolism at the end of 7 days' treatment with dapagliflozin or placebo

	Dapagliflozin	Placebo	<i>P</i>
Baseline C-peptide concentration (nmol/L)	0.10 ± 0.02	0.10 ± 0.03	0.470
Glucose concentration at baseline (mmol/L)	5.43 ± 0.14	5.74 ± 0.17	0.180
Glucose concentration AUC _{0–180} (mmol/L · min)	1,450 ± 70	1,744 ± 87	0.015
Glucose concentration incremental AUC _{0–180} (mmol/L · min)	464 ± 77	720 ± 81	0.026
Glucose R _a at baseline (μmol/min/kg)	13.0 ± 0.77	11.7 ± 0.7	0.009
Glucose R _a AUC _{0–180} (μmol/kg)	3,337 ± 271	2,969 ± 216	0.157
Glucose R _a incremental AUC _{0–180} (μmol/kg)	879 ± 180	758 ± 127	0.596
Glucose R _d at baseline (μmol/min/kg)	13.1 ± 0.7	11.8 ± 0.6	0.009
Glucose R _d AUC _{0–180} (μmol/kg)	2,728 ± 222	2,014 ± 147	0.004
Glucose R _d incremental AUC _{0–180} (μmol/kg)	593 ± 140	275 ± 100	0.049
Glucose MCR at baseline (mL/min/kg)	2.43 ± 0.14	2.06 ± 0.06	0.008
Glucose MCR AUC _{0–180} (mL/kg)	347 ± 26	215 ± 9	<0.001
Glucose MCR incremental AUC _{0–180} (mL/kg)	−38.9 ± 8.1	−78.8 ± 8.2	<0.001
Glycerol concentration at baseline (μmol/L)	81.6 ± 24.2	75.1 ± 26.9	0.392
Glycerol concentration AUC _{0–180} (μmol/L · min)	15,279 ± 1,748	14,684 ± 1,388	0.430
Glycerol concentration incremental AUC _{0–180} (μmol/L · min)	3,412 ± 574	3,524 ± 549	0.801
Glycerol R _a at baseline (μmol/min/kg)	2.71 ± 0.46	2.17 ± 0.28	0.134
Glycerol R _a AUC _{0–180} (μmol/kg)	585 ± 77	543 ± 56	0.048
Glycerol R _a incremental AUC _{0–180} (μmol/kg)	66.3 ± 15.0	98.2 ± 22.5	0.129
BOHB concentration at baseline (mmol/L)	0.39 ± 0.11	0.16 ± 0.05	0.044
BOHB concentration AUC _{0–180} (mmol/L · min)	149 ± 26	117 ± 18	0.035
BOHB concentration incremental AUC _{0–180} (mmol/L · min)	79.6 ± 13.3	88.1 ± 15.4	0.588
NEFA concentration at baseline (mmol/L)	0.61 ± 0.09	0.47 ± 0.07	0.054

Data are means ± SEM. Boldface type indicates statistical significance where *P* < 0.05.

were lower with dapagliflozin versus placebo (0.61 ± 0.06 vs. 1.66 ± 0.48 mmol/L, *P* = 0.0095, and 24.1 ± 0.4 vs. 24.9 ± 0.30 mmol/L, *P* = 0.028, respectively).

During Insulin Withdrawal

BOHB AUC_{0–180 min} was higher with dapagliflozin compared with placebo (mean ± SEM 149 ± 26 vs. 117 ± 18 mmol/L · min, *P* = 0.035). NEFA, venous bicarbonate, pH, and capillary ketones and urinary ketones were not statistically different, but there was a small but significantly higher AUC_{0–180 min} glycerol R_a.

BOHB AUC_{0–180 min} and glycerol R_a AUC_{0–180 min} were negatively correlated with BMI with both dapagliflozin (*r* = −0.58, *P* = 0.048, and *r* = −0.625, *P* = 0.030 respectively) and placebo (*r* = −0.77, *P* = 0.003, and *r* = −0.757, *P* = 0.004) (Supplementary Fig. 1).

Counterregulatory Hormones

At arrival (−120 min), glucagon was significantly higher for the dapagliflozin treatment group compared with that in the placebo group (mean ± SEM 42.1 ± 3.8 vs. 35.2 ± 3.9 ng/L,

P = 0.0127). Insulin at arrival was not different between dapagliflozin and placebo (231 ± 40 vs. 264 ± 50 pmol/L). Glucagon-to-insulin ratio at arrival was also not significantly different between dapagliflozin and placebo (0.24 ± 0.04 vs. 0.19 ± 0.02 ng/pmol).

At 0 min, neither insulin (mean ± SEM 214 ± 45 vs. 235 ± 46 pmol/L) nor glucagon (35.8 ± 2.5 vs. 31.2 ± 3.8 ng/L) was different between dapagliflozin and placebo, respectively, but the glucagon-to-insulin ratio was higher with dapagliflozin (0.27 ± 0.06) than placebo (0.16 ± 0.02 ng/pmol, *P* = 0.04).

During insulin withdrawal, insulin, glucagon, and the glucagon-to-insulin ratio were not significantly different in the two treatment arms (Supplementary Fig. 2).

There was no statistical difference in growth hormone concentration or cortisol concentration at any time point.

Subgroup Analysis

In people with BMI <27 kg/m² vs. BMI >27 kg/m², there was no significant difference in Hb_{A1c} (61 ± 1.2 vs. 59 ± 1.6 mmol/mol) or duration of diabetes

(18.7 ± 5.4 years vs. 29.8 ± 5.6 years, respectively) (Supplementary Table 2).

Baseline glucose R_a, baseline BOHB, and glycerol R_a AUC_{0–180 min} were higher in the low-BMI group with dapagliflozin than in the high-BMI group on placebo (*P* = 0.001, *P* = 0.019, and *P* = 0.012, respectively). These measurements were not different in the high-BMI group with dapagliflozin versus the high-BMI group on placebo (Table 2). NEFA concentrations were not different between dapagliflozin versus placebo in either group (Supplementary Table 2).

For the placebo group, baseline insulin concentration was significantly higher in the high-BMI vs. low-BMI group (*P* = 0.046) (Table 2).

For both the dapagliflozin and placebo groups, NEFA concentration at baseline (both *P* < 0.05) (Supplementary Table 2) and BOHB AUC_{0–180 min} (*P* < 0.01, *P* < 0.05) (Table 2) were significantly lower in the high-BMI versus low-BMI group (Supplementary Table 2).

CONCLUSIONS

This study provides clear evidence that when dapagliflozin was used as an adjunct

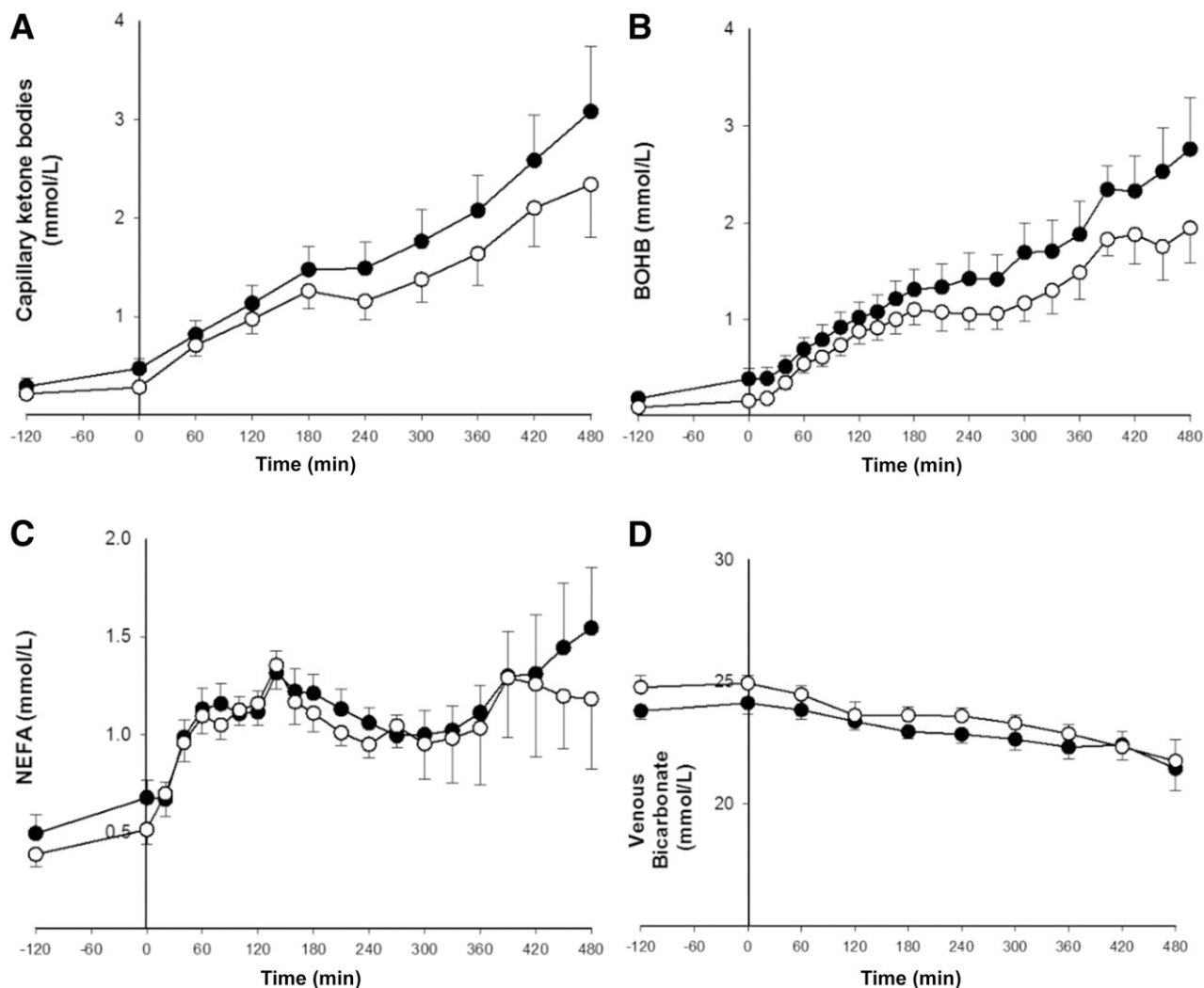


Figure 2—Paired concentration of capillary ketones (A), BOHB (B), and NEFA (C) in plasma, and venous blood bicarbonate (D). All subjects completed 180 min of each metabolic study ($n = 12$). By 480 min, $n = 5$. \circ , placebo; \bullet , dapagliflozin.

therapy in people with type 1 diabetes on insulin pump therapy, plasma BOHB was higher in the dapagliflozin group versus placebo group both in the presence of insulin treatment and during insulin withdrawal. The power of this study from the clinical perspective was the crossover design with each individual undergoing an identical insulin withdrawal protocol and the only difference being the presence or absence of the SGLT2 inhibitor.

The primary end point was plasma glucose concentration at the end of study. This was significantly lower during dapagliflozin treatment than for the placebo group, while the time to study termination did not differ between the two treatments. It is therefore reassuring from a clinical perspective that there was no difference in the time to rescue with

dapagliflozin versus placebo. However, this highlights that glucose levels in the presence of dapagliflozin may not be used as a marker for insulin deficiency in patients in the context of patient-guided “sick day rules.”

It has been hypothesized that SGLT2 inhibition with single-dosing empagliflozin in people with type 2 diabetes results in a reduction in insulin secretion and an augmented glucagon response, which in turn enhances gluconeogenesis and lipolysis (1). The higher baseline glucose R_a with dapagliflozin, in the current study, may be due to augmented gluconeogenesis caused by the increased glucagon/insulin. Another possibility is that it may be due to the increase in glucose R_d , although the study cannot determine whether the increase in glucose R_d is due to increased glucose excretion only or

also due to an increase of tissue glucose uptake stimulated by dapagliflozin.

Although at baseline there was no difference in glycerol R_a (a measure of lipolysis), NEFA concentration was higher with dapagliflozin. The strong relationship between NEFA and BOHB at baseline and during insulin withdrawal suggests that NEFA flux to the liver was driving the higher BOHB with dapagliflozin. When we divided the subjects based on BMI, this effect was only seen in those with a low BMI; the BOHB response in the presence of dapagliflozin at baseline and the rise in glycerol R_a during insulin withdrawal was higher than placebo. This was not seen in the high-BMI group. There was a striking negative relationship between lipolysis and BMI and ketone levels and BMI. While this is only a small group and we must highlight that the

Table 2—Baseline AUC and incremental AUC from 0 to 180 min for glucose, glycerol, and BOHB metabolism at the end of 7 days' treatment with dapagliflozin or placebo in BMI subgroups

	Dapagliflozin		Placebo		BMI <27 kg/m ² vs. placebo P		BMI >27 kg/m ² vs. placebo P	
	BMI <27 kg/m ²	BMI >27 kg/m ²	BMI <27 kg/m ²	BMI >27 kg/m ²	BMI <27 kg/m ²	BMI >27 kg/m ²	BMI <27 kg/m ²	BMI >27 kg/m ²
Age (years)	34.4 ± 1.9	49.4 ± 1.5§	34.6 ± 1.9	49.4 ± 1.5§				
BMI (kg/m ²)	22.5 ± 0.4	31.4 ± 0.3**	22.9 ± 0.4	31.4 ± 0.4**				
Glucose concentration at baseline (mmol/L)	5.4 ± 0.1	5.5 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	0.177	0.694		
Glucose concentration AUC ₀₋₁₈₀ (mmol/L · min)	1,420 ± 43	1,498 ± 30	1,827 ± 53	1,651 ± 44	0.019	0.360		
Glucose concentration incremental AUC ₀₋₁₈₀ (mmol/L · min)	415 ± 46	535 ± 34	778 ± 45	648 ± 51	0.024	0.414		
Glucose R _a at baseline (μmol/min/kg)	14.5 ± 0.3	11.0 ± 0.3†	12.5 ± 0.4	10.5 ± 0.2	0.001	0.528		
Glucose R _a AUC ₀₋₁₈₀ (μmol/kg)	3,524 ± 161	3,076 ± 125	3,250 ± 116	2,579 ± 98	0.428	0.235		
Glucose R _a incremental AUC ₀₋₁₈₀ (μmol/kg)	790 ± 106	1,002 ± 94	978 ± 53	529 ± 106	0.631	0.175		
Glucose R _d at baseline (μmol/min/kg)	14.6 ± 0.3	11.05 ± 0.3†	12.7 ± 0.4	10.6 ± 0.2	0.001	0.521		
Glucose R _d AUC ₀₋₁₈₀ (μmol/kg)	3,000 ± 126	2,347 ± 76	2,230 ± 76	1,692 ± 27	0.009	0.175		
Glucose R _d incremental AUC ₀₋₁₈₀ (μmol/kg)	635 ± 87	546 ± 56	279 ± 42	50 ± 16	0.378	0.037		
Glucose MCR at baseline (mL/min/kg)	2.7 ± 0.1	2.0 ± 0.1†	2.22 ± 0.1	1.9 ± 0.0	0.002	0.285		
Glucose MCR AUC ₀₋₁₈₀ (mL/kg)	389 ± 12	286 ± 7†	228 ± 4	195 ± 3	<0.001	0.007		
Glucose MCR incremental AUC ₀₋₁₈₀ (mL/kg)	-35.9 ± 4.9	-42 ± 4.0	-99.3 ± 10.8	-92 ± 7.0	0.039	0.002		
Glycerol concentration at baseline (μmol/L)	112.9 ± 14.2	37.9 ± 4.2	104.5 ± 16.5	33.9 ± 2.9	0.426	0.752		
Glycerol concentration AUC ₀₋₁₈₀ (μmol/L · min)	17,428 ± 1,007	12,270 ± 525	16,477 ± 771	12,174 ± 491	0.396	0.876		
Glycerol concentration incremental AUC ₀₋₁₈₀ (μmol/L · min)	3,624 ± 300	3,115 ± 403	3,185 ± 239	3,999 ± 460	0.455	0.212		
Glycerol R _a at baseline (μmol/min/kg)	3.41 ± 0.23	1.73 ± 0.20§	2.60 ± 0.12	1.57 ± 0.17	0.118	0.648		
Glycerol R _a AUC ₀₋₁₈₀ (μmol/kg)	6.94 ± 4.1	433 ± 31§	637 ± 27	411 ± 24	0.012	0.866		
Glycerol R _a incremental AUC ₀₋₁₈₀ (μmol/kg)	59.7 ± 5.8	75.8 ± 13.8	107.5 ± 12.7	85.1 ± 13.5	0.119	0.661		
BOHB concentration at baseline (mmol/L)	0.6 ± 0.05	0.10 ± 0.03†	0.23 ± 0.06	0.06 ± 0.01	0.019	0.703		
AUC ₀₋₁₈₀ BOHB concentration (mmol/L · min)	196 ± 11	84 ± 12†	156 ± 6	62 ± 7§	0.071	0.273		
BOHB concentration incremental AUC ₀₋₁₈₀ (mmol/L · min)	89 ± 7	66 ± 8	114 ± 8	52 ± 6§	0.219	0.521		
Insulin concentration (pmol/L) at baseline	154 ± 22	298 ± 25	159 ± 11	341 ± 39§	0.316	0.924		

Data are means ± SEM. Boldface type indicates statistical significance where *P* < 0.05. Significance between BMI groups: §*P* < 0.05, †*P* < 0.01, ***P* < 0.001.

study design was not powered to look at the difference in BMI, our results suggest a greater risk of ketosis in people with type 1 diabetes with a low BMI, which may be of clinical relevance. Although baseline NEFA concentration was higher with dapagliflozin, there was no difference in NEFA concentration with dapagliflozin in the whole group and the BMI subgroups following insulin withdrawal. However, it is possible that an increase in NEFA production was matched by an increase in NEFA clearance.

Individuals with a BMI >27 kg/m² also required greater insulin doses and had higher plasma insulin concentrations than people with a BMI <27 kg/m², leading to lower plasma level of ketones. This can be clinically important because it suggests that the greater insulin requirement in people with a BMI >27 kg/m² is likely to minimize the risk of ketoacidosis caused by SGLT2 therapy.

While an increase in ketogenesis driven by lipolysis is one mechanism for the rise in BOHB, the rise in lipolysis with dapagliflozin was small and we cannot rule out the possibility that reduced ketone uptake by peripheral tissues or reduced urinary excretion of ketones may also play a role. The excretion of ketones occurs by a balance between glomerular filtration and tubular reabsorption. Experimental and clinical data have established that SGLT2 inhibition can reduce glomerular filtration rate in people with type 1 and 2 diabetes, thereby reducing renal ketone excretion (19). In the current study, although plasma ketone concentrations were higher during dapagliflozin treatment, urinary ketone concentrations were not different versus the placebo group. This contrasts with a study in people with type 2 diabetes that showed increased clearance and fractional urinary excretion of BOHB after single and 4-week chronic use of empagliflozin (20).

In the presence of prolonged insulin withdrawal secondary to periods of illness or stress, there may also be upregulation of the renal reabsorption capacity of ketones, but this needs further investigation. This also has clinical implications, as historically ketonuria has been used to screen for the presence of ketosis (21).

It has been suggested that short-term fasting and dehydration predispose people with type 1 diabetes to develop euglycemic DKA during periods of insulin withdrawal/deficiency (19). SGLT2

inhibitors may by nature of their mechanism of action further predispose a person to the risks of euglycemic DKA. SGLT2 inhibitors lower the glucose availability during insulin deficiency by encouraging glucose excretion and fluid loss through persistent glycosuria. The base excess concentration at baseline (mmol/L) was statistically higher for the dapagliflozin group, indicating a greater need to balance acid to ensure a normal body pH with administration of SGLT2 inhibitors. The SGLT2 inhibitor was only taken for a short term, and further evidence is required to establish any longer-term adaptations with administration on a regular basis.

In summary, this study provides evidence that when dapagliflozin is used as an adjunct therapy in people with type 1 diabetes on insulin pump therapy, there is a rise in ketones during insulin deficiency. This has clinical implications. The stability of glucose levels indicates that there will need to be more reliance on capillary ketone monitoring with SGLT2 inhibitors to prevent this predictable and preventable risk (22). Emphasis should be on ensuring adequate basal insulin levels and avoiding calorie restriction and dehydration. The patient and clinician will need to be constantly vigilant, and there must be a low threshold for stopping this class of drug during periods of illness or intercurrent stress (23). A four-step approach to address the risk of DKA during treatment with SGLT2 inhibitors in people with type 1 diabetes has been suggested as a lifesaving measure to help clinicians and patients (24). In addition, an international consensus has been published to address the risk management of DKA in people with type 1 diabetes (25). SGLT2 inhibitors are now being used in clinical practice both within and outside of license, and we watch with interest the clinical benefits and risks of this class of drug as more real-world experience is gained in people with type 1 diabetes.

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Author Contributions. R.A.H., F.S.-M., and M.S. drafted the manuscript. F.S.-M. and R.G. carried out the metabolic studies. F.S.-M. carried out sample analysis and interpreted data. N.J. assisted with sample analysis. B.A.F., A.M.U., M.D., and D.L.R.-J. participated in the design of the study, interpreted data, and reviewed and edited the manuscript. A.M. and S.J. completed the statistical analysis. R.A.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. **Prior Presentation.** Parts of this study were presented in abstract form at the 55th Annual Meeting of the European Association for the Study of Diabetes, Barcelona, Spain, 16–20 September 2019.

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