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Title page

Full Title Metabolic effects of an SGLT2 inhibitor (dapagliflozin) during a period of acute insulin withdrawal and development of ketoacidosis in people with type 1 diabetes.

Running Title: SGLT2 inhibitor in type 1 diabetes

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Abstract

Objective: To determine the effect of $SGLT_2$ 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 wash out 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 rate of glucose production (Ra), disappearance (Rd) and lipolysis. At isotopic steady state insulin was withdrawn and the study terminated after 600 minutes or earlier if blood glucose reached 18mmol/L, bicarbonate <15mmol/L, venous pH <7.35 or capillary ketones >5.0 mmol/L.

Results: At baseline, glucose Ra was significantly higher with dapagliflozin than placebo. Following insulin withdrawal, plasma glucose concentrations at the end point were significantly lower with dapagliflozin than placebo and $AUC_{0\text{-}180min}$ glucose Rd and $AUC_{0\text{-}180min}$ β-hydroxybutyrate (BOHB) were significantly higher. There was a small but significantly higher $AUC_{0\text{-}180min}$ glycerol Ra (measure of lipolysis) with dapagliflozin. Non-esterified fatty acid concentrations were not different between treatments.

When divided by BMI>27 and <27kg/m^2, basal glucose Ra and BOHB, and $AUC_{0\text{-}180min}$ glycerol Ra were significantly higher in the low BMI group with dapaglifozin versus placebo treatment.

Conclusions: During insulin withdrawal the increase in BOHB with

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

$SGLT_2$ 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 $SGLT_2$ inhibitor class may have beneficial effects in the management of type1 diabetes. Phase 2 and 3 clinical trial studies have shown encouraging results with lower HbA1c levels, 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 DEPICT-1 diabetic ketoacidosis (DKA) occurred in 1% and 2% of the 5mg and 10mg dapagliflozin groups respectively (2). Pooled data from the 26-week EASE-2 and EASE-3 studies showed DKA occurred in 0.8%, 3.3% and 4.3% of the empagliflozin 2.5mg, 10mg and 25mg arm respectively and 1.2% in the placebo arm (4). In Tandem-1 euglycemic ketoacidosis occurred in 3.4% and 4.2% of the 200mg and 400mg sotagliflozin groups and 0.4% in the placebo group over 24 weeks (6). Finally, in an 18-week randomised study with canagliflozin, ketone related adverse events occurred in 5.1% of the 100mg arm and 9.5% of the 300mg arm (7).

The FDA have not approved $SGLT_2$ 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 inter-current 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 body mass index greater than 27kg/$m^{2.}$ (9) and received evidence-based recommendation by National Institute for Clinical Excellence (NICE) in August 2019 (10).

The incidence of DKA is between 0 – 56 cases per 1000 person – years amongst people with type1 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 hyperglycaemia. Lipolysis is increased due to lack of insulin's action on hormone sensitive lipase resulting in increased release of non-esterified fatty acids (NEFA) and glycerol from adipose triglyceride stores (12). In the liver, due to the diversion of oxaloacetate towards gluconeogenesis, there is a reduced capacity of the Krebs cycle to oxidise acetyl CoA derived from the β oxidation of NEFA. Consequently, there is an increased flux of acetyl CoA towards ketogenesis giving rise to increased acetoacetate, BOHB and acetone (13). Hence, in insulin deficiency, there are two parallel metabolic processes: glucose over-production and ketone over-production.

The potentially life-threatening state of euglycemic DKA is unusual and was first described in 1973 (14). A speculative mechanism for its development with $SGLT_2$ inhibitors is that the insulin independent removal of glucose from the body enables glycemic control concurrent with either an absolute or relative deficiency of insulin. In addition, ketoacidosis may be driven by an increase in counter regulatory hormones i.e. glucagon, cortisol or growth hormone. Using stable isotope techniques, this study explored these potential mechanisms by studying the effect of $SGLT_2$ inhibitor, dapagliflozin on glucose flux, lipolysis and ketone body concentration during hyperglycaemia in people with absolute endogenous insulin deficiency.

RESEARCH DESIGN AND METHODS

A single centre investigator led, double-blind placebo controlled cross over study with a 4-week wash out period was performed in patients with type 1 diabetes using insulin pump therapy. Ethics approval was granted from HRES committee: Southern Central Berkshire B. The clinical trial was registered with the European Clinical Trials Database (EudraCT) under the number 2015-002094-38. The study was funded by Diabetes UK and Investigational Medicine Product (IMP) provided by Astra Zeneca Ltd.

Participants with type 1 diabetes for greater than 12-months were recruited between February 2018 – October 2018 using the diabetes insulin pump database at the Royal Surrey County NHS Hospital. Type 1 diabetes was established by clinical presentation, treatment response and C-peptide level. Exclusion criteria included proliferative retinopathy requiring acute treatment within last three months, moderate to severe renal impairment (creatinine clearance [CrCl] < 60 mL/min or estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2, severe hepatic impairment,

NYHA class III-IV cardiac failure, uncontrolled cardiac arrhythmia, uncontrolled hypertension, mental incapacity, pregnancy or breast feeding, those of child-bearing potential not taking adequate contraception precautions and suspected allergy to trial products.

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), hypoglycaemia frequency (capillary glucose level <4mmol/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 22:00 hours the day before. They were also asked not to undertake any strenuous exercise or consume alcohol for 24h before the study day. All participants were using continuous subcutaneous insulin therapy and disconnected their insulin pumps at 06:00 hours on the morning of a metabolic study. They were transferred to a soluble variable insulin infusion to maintain a whole blood concentration glucose at 5 mmol/L. [6,6-$^{2}H_{2}$]glucose and [1,1,2,3,3 $^{5}H_{2}$]glycerol were infused from -120 min to the study endpoint to measure glucose Ra and Rd and lipolysis respectively (Cambridge isotopes, CK Gas Products Ltd, Ibstock, UK).

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 allowed to increase.

Blood samples were taken to measure plasma glucose and glycerol enrichment and concentration, and concentrations of non-esterified fatty acid (NEFA) and plasma BOHB every 20 minutes until 180 minutes and then at 30 minute 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 minute intervals. Urine samples were collected over 2 hour intervals for measurement of glucose and spot urinary ketones.

Rescue and study termination

At 600 minutes (10 hours) or in the event of blood glucose of 18mmol/L, bicarbonate <15mmol/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 stabilised.

Plasma measurements

On the study day plasma glucose concentration was measured using a glucose oxidase technique on a glucose analyser (YSI 2300 Clandon Scientic, Yellow Springs Instruments, Yellow Springs, OH, USA). Whole-blood samples were immediately centrifuged, and aliquots of plasma stored at -80^{o}C to be analysed at a later date in a laboratory setting.

Plasma glucose concentrations were measured with a Roche Cobas MIRA analyser using the ABX Pentra glucose kit (Horiba ABX, Northampton, UK) and plasma glycerol and BOHB concentrations using Randox kits (Glycerol and RANBUT; Randox Laboratories, Co. Antrim, UK). Plasma NEFA concentrations were measured using an enzymatic kit from (WAKO Chemicals GmbH, Neuss Germany).

Insulin and glucagon were measured using radioimmunoassays purchased from Merck Millipore, Merck Chemicals, Nottingham, UK). Serum cortisol concentration were measured using an in-house radioimmunoassay. Serum Growth hormone concentrations were measured using an immunoradiometric kit purchased from DRG Instruments GmbH, supplied by IDS, Tyne and Wear, UK. The isotopic enrichment of plasma glucose was determined as the trimethylsilyl-O-methyloxime derivative (15) using gas chromatography-mass spectrometry (model 5975 CMSD inert XL EI/CI MSD; Agilent Technologies, Wokingham, UK). 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 rate of appearance (Ra), rate of disappearance (Rd) and glycerol production were calculated using Steele's 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 minutes 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, each studied with and without the SGLT2 inhibitor and a SD of 20% 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 analysed as the response variable in a general linear mixed model (using PROC MIXED procedure in SAS software), with treatment, period, 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 denominator degrees of freedom were adjusted using the Kenward-Roger approximations.

The secondary endpoints 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, 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 analysed as per the primary endpoint.

In order to assess the impact of participant BMI level on study conclusions the participants were classified as ‘high BMI’ (BMI > 27kg/m^2, N = 5) or ‘low BMI’ (BMI < 27kg/m^2, N = 7). The data for which a single value per participant per period was analysed were then re-analysed as above with the modification that the model independent variables included, additionally, the BMI classification and the interaction of the BMI classification with treatment. The estimate of- and p-value for-the treatment effect at each separate level of BMI the same information for the effect

of BMI at each separate treatment were reported in addition to the results reported above.

The data for which several time point values per participant per period were analysed were then re-analysed as 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 and 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 & 8 female) with type 1 diabetes, duration of diabetes 23.3±4.1 y (age 40.7±3.9 years, BMI 26.8±1.4, HbA1C 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 v placebo arm respectively with duration of type 1 diabetes of 17y).

During the seven day treatment period there was no significant difference in insulin dose between dapagliflozin v placebo (0.056±0.007 U/kg vs 0.058±0.008 U/kg,

respectively). Two participants reduced their basal setting due to hypoglycaemia. Other participants did not change their insulin dose but encountered more hypos overnight which were remedied by taking Gluco gel (hypoStop gel).

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

Glucose Concentration (Figure 1)

At 0 min, glucose concentration was not different between treatments. 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 glucose concentration was 8.5 ± 0.7mmol/L with dapagliflozin treatment and 14.3 ± 1.1 mmol/L with placebo (p=0.0005) (Figure 1). Urinary glucose excretion between 0-120 min (n=6) was 5.10 ± 0.80 micromol/kg/min with dapagliflozin and 0.029 ± 0.01 micromol/kg/min with placebo (p=0.003).

Glucose Metabolism (Figure 1 and Table 1)

At baseline (0 min), when isotopic enrichment and glucose concentration were in a steady state glucose Ra was significantly higher with dapagliflozin compared with placebo (p=0.0088). At this time point glucose Rd is equal to glucose Ra. During insulin withdrawal glucose Ra increased, peaking at 90 minutes and then declined with no difference in AUC between treatments. $AUC_{0\text{-}180\ min}$ Glucose Rd and MCR were higher with dapagliflozin compared with placebo (p=0.0041, p<0.0001).

BOHB, Non-esterified Fatty Acid and Glycerol Metabolism (Figure 2 and Table 1)

Baseline. There was no difference in baseline glycerol Ra (a measure of lipolysis) between treatments. Baseline BOHB was higher with dapagliflozin (0.39±0.11 vs 0.16±0.05 mmol/L, p=0.044) and NEFA concentration was higher (0.61±0.09 vs 0.47±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 (Supplement figure 3). Base excess concentration and venous bicarbonate were lower with dapagliflozin versus placebo (0.61±0.0.6 vs 1.66 ± 0.48mmol/L, p=0.0095 and 24.1±0.4 vs 24.9±0.30mmol/L, p=0.028 respectively).

During insulin withdrawal. $AUC_{0\text{-}180\ min}$ BOHB was higher with dapagliflozin compared with placebo (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 $AUC_{0\text{-}180\ min}$ glycerol Ra.

$AUC_{0\text{-}180\ min}$ BOHB and $AUC_{0\text{-}180\ min}$ glycerol Ra 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) (Supplemental Figure 1)

Counter Regulatory Hormones

At arrival (-120 min), glucagon was significantly higher with dapagliflozin treatment compared to placebo (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/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 (214 ± 45 vs 235 ± 46 pmol/L) nor glucagon (35.8 ± 2.5 vs 31.2 ± 3.8 ng/L) were different between dapagliflozin and placebo respectively but

the glucagon/insulin ratio was higher with dapagliflozin (0.27 ± 0.06) than placebo (0.16 ± 0.02 ng/pmol, p= 0.04).

During insulin withdrawal neither insulin, glucagon nor the glucagon/insulin ratio were significantly different in the two treatment arms (Supplement Figure 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/m^2 vs BMI>27kg/m^2, 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 y vs 29.8±5.6 y, respectively) (Supplement Table 2)

Baseline glucose Ra, baseline BOHB and $AUC_{0\text{-}180\ min}$ glycerol Ra were higher in the low BMI group with dapaglifozin versus placebo treatment (p=0.001, p=0.019 and p=0.012 respectively). These measurements were not different in the high BMI group with dapaglifozin versus placebo treatment (Table 2). NEFA concentrations were not different between dapagliflozin versus placebo in either group (Supplement Table 2).

When treated with placebo baseline insulin concentration was significantly higher in the high BMI vs low BMI group (p=0.046) (Table 2).

With both dapagliflozin and placebo, NEFA concentration at baseline (both p<0.05, Supplement Table 2) and $AUC_{0\text{-}180\ min}$ BOHB (p<0.01, p<0.05, Table 2) were significantly lower in the high BMI vs low BMI group (Supplement Table 2).

Discussion

This study provides clear evidence that when dapagliflozin was used as an adjunct therapy in people with type 1 diabetes using insulin pump therapy, plasma BOHB was higher versus placebo 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 with the only difference being the presence or absence of a SGLT2 inhibitor. The primary endpoint was plasma glucose concentration at the end of study. This was significantly lower during dapagliflozin treatment than placebo, 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 either dapagliflozin or placebo. However, it highlights that glucose levels in the presence of dapagliflozin may not be used as a marker for insulin deficiency in patients during patient guided ‘sick day rules’.

It has been hypothesised that $SGLT_2$ 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 Ra with dapagliflozin, in the current study, may be due to augmented gluconeogenesis caused by the increased glucagon/insulin. Another possibility, is that it maybe due to the increase in glucose Rd, although the study cannot determine whether the increase in glucose Rd 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 Ra (a measure of lipolysis), NEFA concentration was higher with dapagliflozin. The strong relationship between NEFA and BOHB at baseline and during insulin withdrawal suggests 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 i.e. the BOHB response to dapagliflozin at baseline and the rise in glycerol Ra 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 study design was not powered to look at the difference between BMI, it suggests a greater risk of ketosis in people with type 1 diabetes with a low BMI which maybe of clinical relevance. Although baseline NEFA concentration was higher with dapagliflozin there was no difference in NEFA concentration with dapagliflozin in both the whole group and the BMI subgroups following insulin withdrawal. However, it's possible that an increase in NEFA production was matched by an increase in NEFA clearance.

Individuals with a BMI>27kg/m^2 also required greater insulin doses and had higher plasma insulin concentrations than people with a BMI <27 kg/m^2 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 >27kg/m^2, 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 (21). In the current study, although plasma ketone concentrations were higher during dapagliflozin treatment urinary ketone concentrations were not different from placebo. This contrasts with a study in people with type 2 diabetes which showed increased clearance and fractional urinary excretion of β-hydroxybutyrate after single and 4 week chronic use of empagliflozin (19..

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 (20.).

It has been suggested that short term fasting and dehydration predisposes people with type 1 diabetes to develop euglycemic DKA during periods of insulin withdrawal / deficiency (21). $SGLT_2$ inhibitors may by nature of their mechanism of action further predispose a person to the risks of euglycemic DKA. $SGLT_2$ 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 $SGLT_2$ inhibitors. The $SGLT_2$ inhibitor was only taken for a short term and further evidence is required to establish any longer-term adaptions if administered on a regular basis.

In summary, this study provides evidence that when used as an adjunct therapy in people with type 1 diabetes using 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 in 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 inter-current 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 life saving 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). $SGLT_2$ inhibitors are now being used in clinical practice both within and outside of licence and we watch with interest the clinical benefits and risks of this class of drug as we gain more real-world experience in people with type 1 diabetes.

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Conflict of interests: M.D. has acted as consultant, advisory board member and speaker for Novo Nordisk, Sanofi-Aventis, Lilly, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca and Janssen, an advisory board member for Servier and Gilead Sciences Ltd and as a speaker for NAPP, Mitsubishi Tanabe Pharma Corporation and Takeda Pharmaceuticals International Inc and received grants in

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Author Contributions: R. G. and F. S-M carried out the metabolic studies, R. H., F. S-M, M. S., drafted the manuscript and takes full responsibility for the work as a whole, including the study design, access to data and the decision to submit and publish the manuscript, F. S.-M. carried out, sample analysis and interpreted data. N. J. assisted with sample analysis. M. D., A. M. U., B. F. 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 statistically analysis.

Guarantor Statement: RH takes full responsibility for the work as a whole, including the study design, access to data and the decision to submit and publish the manuscript

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Table 1. Baseline, AUC and incremental AUC from 0-180 min for glucose, glycerol and BOHB metabolism at the end of 7days treatment with dapagliflozin or placebo. Results are mean ± SEM.

	Dapagliflozin	Placebo	P Value
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
AUC_{0-180} glucose concentration (mmol/L*min)	1450±70	1744±87	**0.015**
Incremental AUC_{0-180} glucose concentration (mmol/L*min)	464±77	720±81	**0.026**
Glucose Ra at Baseline (micromol/min/kg)	13.0±0.77	11.7±0.7	**0.009**
Glucose Ra AUC_{0-180} (micromol/kg)	3337±271	2969±216	0.157
Glucose Ra incremental AUC_{0-180} (micromol/kg)	879±180	758±127	0.596
Glucose Rd at Baseline (micromol/min/kg)	13.1±0.7	11.8±0.6	**0.009**
Glucose Rd AUC_{0-180} (micromol/kg)	2728±222	2014±147	**0.004**
Glucose Rd incremental AUC_{0-180} (micromol/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 (micromol/L)	81.6±24.2	75.1±26.9	0.392
Glycerol concentration AUC_{0-180} (micromol/L*min)	15279±1748	14684±1388	0.430
Glycerol concentration incremental AUC_{0-180} (micromol/L*min)	3412±574	3524±549	0.801
Glycerol Ra at Baseline (micromol/min/kg)	2.71±0.46	2.17±0.28	0.134
Glycerol Ra AUC_{0-180} (micromol/kg)	585±77	543±56	**0.048**
Glycerol Ra incremental AUC_{0-180} (micromol/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**
AUC_{0-180} BOHB concentration (mmol/L*min)	149±26	117±18	**0.035**
Incremental AUC_{0-180} BOHB concentration (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

Significance between BMI groups, P value * <0.05; †<0.01, **<0.001

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

	Dapagliflozin BMI <27 kg/m^2	Dapagliflozin BMI >27 kg/m^2	Placebo BMI <27 kg/m^2	placebo BMI >27 kg/m^2	BMI <27 Dapa vs Placebo P Value	BMI > 27 Dapa vs Placebo P Value
Age (y)	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
AUC_{0-180} glucose concentration (mmol/L*min)	1420±43	1498±30	1827±53	1651±44	**0.019**	0.360
Incremental AUC_{0-180} glucose concentration (mmol/L*min)	415±46	535±34	778±45	648±51	**0.024**	0.414
Glucose Ra at Baseline (micromol/min/kg)	14.5±0.3	11.0±0.3†	12.5±0.4	10.5±0.2	**0.001**	0.528
Glucose Ra AUC_{0-180} (micromol/kg)	3524±161	3076±125	3250±116	2579±98	0.428	0.235
Glucose Ra incremental AUC_{0-180} (micromol/kg)	790±106	1002±94	978±53	529±106	0.631	0.175
Glucose Rd at Baseline (micromol/min/kg)	14.6±0.3	11.05±0.3†	12.7±0.4	10.6±0.2	**0.001**	0.521
Glucose Rd AUC_{0-180} (micromol/kg)	3000±126	2347±76	2230±76	1692±27	**0.009**	0.175
Glucose Rd incremental AUC_{0-180} (micromol/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 (micromol/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} (micromol/L*min)	17428±1007	12270±525	16477±771	12174±491	0.396	0.876

Glycerol concentration incremental AUC_{0-180} (micromol/L*min)	3624±300	3115±403	3185±239	3999±460	0.455	0.212
Glycerol Ra at Baseline (micromole/min/kg)	3.41±0.23	1.73±0.20*	2.60±0.12	1.57±0.17	0.118	0.648
Glycerol Ra AUC_{0-180} (micromol/kg)	6.94±41	433±31*	637±27	411±24	**0.012**	0.866
Glycerol Ra incremental AUC_{0-180} (micromol/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
Incremental AUC_{0-180} BOHB concentration (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

Significance between BMI groups, P value * <0.05; †<0.01, **<0.001

Figure 1: Paired plasma glucose concentration (A), Glucose Ra (B) and Glucose Rd (C) and Glycerol Ra (D). All subjects completed 180 minutes of each metabolic study (n=12). By 480 min n=5. Dapagliflozin closed symbols. Placebo open symbols.

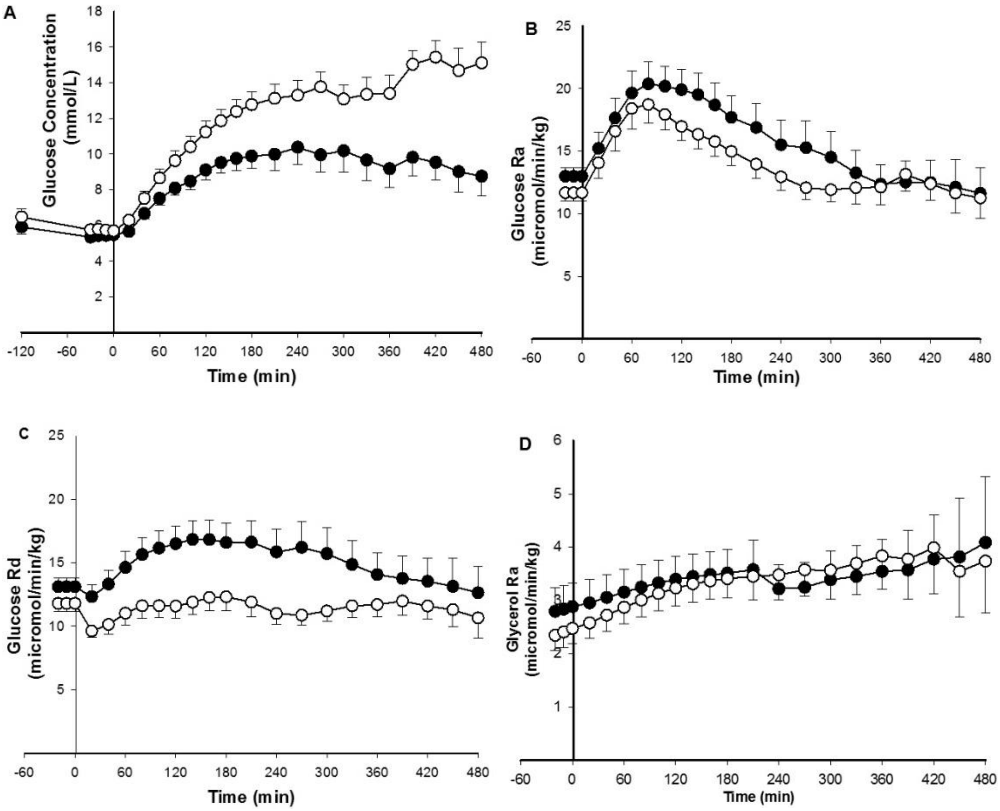

Figure 2: Paired concentration of capillary ketones (A) Beta hydroxybutyrate (B) and NEFA (C) in plasma, and venous blood bicarbonate (D). All subjects completed 180 minutes of each metabolic study (n=12). By 480 min n=5. Dapagliflozin closed symbols and placebo open symbols.