The SGLT2 inhibitor, dapagliflozin increases the oxidation of ingested fatty acids to ketones in type 2 diabetes.

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Running Title: SGLT2 inhibitor increases postprandial ketones

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**Abstract**

**Objective**. To investigate the mechanism for increased ketogenesis following treatment with SGLT2 inhibitor, dapagliflozin in people with type 2 diabetes.

**Research, Design & Methods.** This was a double-blind placebo-controlled crossover study with a 4-week washout period. Participants received dapagliflozin or placebo in random order for 4 weeks. After each treatment, they ingested 30ml of olive oil containing [U-13C] palmitate to measure ketogenesis with blood sampling for 480 min. Stable isotopes of glucose and glycerol were infused to measure glucose flux and lipolysis respectively at 450-480 min.

**Results.** Glucose excretion rate was higher and peripheral glucose uptake lower with dapagliflozin than placebo. Plasma beta-hydroxybutyrate (BOHB) concentrations and [13C2] BOHB concentrations were higher and glucose concentrations lower with dapagliflozin than placebo. Non-esterified fatty acids (NEFA) were higher with dapagliflozin at 300 and 420 min but lipolysis at 450-480 min was not different. Triacylglycerol (TAG) at all time points and endogenous glucose production rate at 450-480 min were not different between treatments.

**Conclusions.** The increase in ketone enrichment from the ingested palmitic acid tracer suggests meal derived fatty acids contribute to the increase in ketones during treatment with dapagliflozin. The increase in BOHB concentration with dapagliflozin, occurred with only minimal changes in plasma NEFA concentration and no change in lipolysis. This suggests a metabolic switch to increase ketogenesis within the liver.

**Introduction**

SGLT2 inhibitors are a class of drug that promote urinary glucose excretion to lower blood glucose levels. In type 2 diabetes inadequately controlled with metformin, dapagliflozin as monotherapy (1) and when added to metformin improved glycaemic control (2,3), reduced weight and resulted in less hypoglycemia than glipizide (4). In people whose type 2 diabetes mellitus was inadequately controlled with insulin therapy, adding dapagliflozin reduced HbA1c levels and weight over 48 weeks without increasing overall insulin dose (5).

The observation that ketogenesis or episodes of euglycaemic ketoacidosis are of higher frequency in people with type 1 and type 2 diabetes on SGLT2 inhibitors, is of considerable worldwide interest (6;7). Ferrannini et al (7) have shown in type 2 diabetes that, after 4 weeks treatment with the SGLT2 inhibitor, empagliflozin, lipid oxidation rate increased during a meal test. This suggests that the homeostatic response to losing glucose by urinary excretion is to switch metabolism in favour of non-esterified fatty acids (NEFA). Animal models of type 2 diabetes and heart failure have shown treatment with SGLT2 inhibitors increase exercise induced ketogenesis and fatty acid oxidation (8;9). In a previous study we found treatment with dapagliflozin in type 1 diabetes following insulin withdrawal, increased BOHB concentration by 27 % despite no increase in NEFA concentrations and only a small increase in lipolysis (10). This suggested mechanisms additional to increased delivery of fatty acids to the liver may drive ketogenesis with SGLT2 inhibitors.

In this study the contribution of ingested fatty acids to ketogenesis was investigated using tracer labelled olive oil following 28 days administration of dapagliflozin in people with type 2 diabetes. In addition, stable isotopes of glucose and glycerol were infused to measure glucose flux and lipolysis respectively.

**Research Design and** **Methods**

The study was a randomised double-blind placebo control cross over trial of people with type 2 diabetes at the Cedar Centre, Royal Surrey NHS Foundation Trust, UK. 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 2016-004878-17 and Clinical Trials.gov NCT 04219124. The study was funded Astra Zeneca Ltd. Inclusion criteria were diagnosis of type 2 diabetes >12 months, single, dual or triple therapy glucose lowering agents comprising of sulphonylureas, biguanides and DDP-IV but no previous exposure to SGLT2 inhibitors, aged 18 – 75 years, BMI < 40 kg/m2, HbA1c of ≥ 6.5% (48 mmol/mol) and < 9% (75 mmol/mol) within 1 month of screening. Exclusion criteria included proliferative retinopathy requiring acute treatment within the previous three months, moderate to severe renal impairment (creatinine clearance [CrCl] < 60 mL/min or estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m2, 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.

***Outcomes and Endpoints***

## The primary outcome was the effect of dapagliflozin compared to placebo on plasma 3-hydroxybutyrate concentration. Secondary outcomes included the effect of dapagliflozin compared with placebo on hepatic ketone body metabolism, whole body lipolysis, glucose metabolism and the metabolome.

The primary endpoint was plasma BOHB concentration at the end of the meal study. Secondary endpoints were glucose production and disposal rate, glycerol production rate, palmitate enrichment in plasma NEFA and TAG, 3-hydroxybutyrate enrichment, glucagon and insulin concentrations and plasma acylcarnitines measured by targeted metabolomics.

***Design***

Participants gave written informed consent. Following screening and randomisation

participants received either 10mg daily dapagliflozin or placebo for 28 days prior to the metabolic study visit. After a 28-day washout period they switched to the other treatment (Supplement Figure S1). They were made aware of potential changes in glycaemic control and were asked to record trial medication administration, any concomitant medication, hypoglycaemia frequency (capillary glucose level <4 mmol/L), fasting ketone levels and any adverse events.

***Metabolic study***

Participants were asked to refrain from drinking alcohol and strenuous exercise for 24 hours prior to the metabolic assessment day. Participants were given a standardised 500-kcal meal the evening before the test and were asked to fast overnight. They were asked to return their unused Investigational Medicinal Product (IMP) at this visit. Intravenous cannulae were inserted into the antecubital fossa of each arm: one for taking blood samples and the other for the infusion of the glucose and glycerol isotopes. At 0 min, the study medication was taken and a stable isotope of [U-13C] palmitate (200 mg; 98% enriched, CK isotopes Ltd, Leicestershire, UK) in 30 ml virgin olive oil and a few drops of lemon juice (to make it palatable) was ingested in less than 2 min. Dilution of the [U-13C] palmitate with palmitate in olive oil resulted in a tracer to tracee ratio (TTR) of 0.0384. Fasting blood samples were taken at -10 and 0 min to measure baseline enrichments and concentrations and then samples were taken at regular intervals until 480 min to measure the concentrations of NEFA, BOHB, TAG, LDL-cholesterol, insulin, glucagon, as well as stable isotope enrichment and concentration in plasma TAG, NEFA and BOHB. An additional blood sample was taken at 480 min for targeted metabolomics. A primed infusion of [6,6-2H2] glucose and [1,1,2,3,3-2H5] glycerol was administered from 360 to 480 min and the concentration and enrichment of both glucose and glycerol measured from 450 to 480 min. Urine was collected to measure glucose excretion at 120, 360, 450 and 480 min. At the end of the study participants were prescribed the IMP or placebo required for the second arm of the study depending on the randomisation code, to be taken after the washout period. The metabolic study was repeated after 28 days treatment.

***Plasma measurements***

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). Plasma total cholesterol, LDL cholesterol and TAG concentrations were measured using enzymatic calorimetric kits; the ABX Pentra, CholesterolCP, LDL direct CP and the ABX Pentra Triglyceride CP, respectively (Horiba ABX, Northampton, UK).

Glucagon concentrations were measured using radioimmunoassay’s purchased from Merck Millipore, Merck Chemicals, Nottingham, UK). Insulin concentration was measured using and ELISA 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 (11) 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 (12) using a gas chromatography-mass spectrometry model 5975 network MSD (Agilent Technologies).

The isotopic enrichment of [U-13C] palmitate was determined in the fatty acid methyl ester (FAME) moieties in plasma TAG and non-esterified fatty acids. It was possible to determine the concentration of the palmitate in NEFA and TAG by addition of internal standards of heptadecanoic acid and triheptadecanoate to the samples before extraction. Extraction was by the Folch method and TAG and NEFA were purified by thin layer chromatography, visualised by 8-anilino-1-napthalene-sulfonic acid in water. The scraped TAG and NEFA spots were collected into tubes and solubilised with toluene and esterified using sulphuric acid in methanol (6%) at 80oC for 2h. The samples were then neutralised and extracted into hexane. FAMEs in hexane were analysed by gas-chromatography isotope ratio mass spectrometry (GC-C-IRMS, Thermo).

The isotopic enrichment of BOHB was determined as tert-butyldimethylsilyl (di-tTBDMS) derivative of BOHB(13). Blood taken on the day of study in cooled fluoride/EDTA containing tubes were centrifuged immediately at 1600 g for 10 min at 4oC. Plasma (2ml) was then deproteinised with cooled 2ml perchloric acid (PCA, 10% w/v) mixed vigorously, and stored at -80oC until analysis. On the day of analysis, the PCA containing samples were thawed on ice and centrifuged at 1600 g for 20 min at 4oC. The supernatant was transferred to a new tube and neutralised by dropwise addition of neutralizing agent, potassium bicarbonate/potassium carbonate (1.5M) and mixing after each drop to release trapped CO2 in the solution during neutralisation step. The volumes of the sample were recorded pre and post neutralisation. The sample was then centrifuged to eliminate the salt formed and the pH was checked to be between pH 7-8. Following the reacidification of the sample (0.5 ml) to pH 1 with HCl (1M) the metabolites were extracted with ice cold mixed solution of ethylacetate : diethylether (1:1; v/v). vortexed for 15 min and centrifuged at 710 g for 20 min at 4oC. The solvent layer was transferred to another tube and derivatised by adding pyridine (20 µl) and MTBSTFA containing 1% TBDMCS (20µl) overnight at room temperature in the dark. The solvent was evaporated, and the sample reconstituted in decane for analysis by GC-MS (model 5973 Agilent Technologies).

***Targeted metabolomics***

Acylcarnitines were measured by LC/MS (Waters Aquity UPLC/Waters Xevo TQ-S mass spectrometer, Milford MA, USA) using the Absolute/DQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria). Plasma samples were measured in duplicate. A total of 40 acylcarnitines were measured. Any which had > 25% of concentrations below the limit of detection were excluded. Samples were run on 96-well plates in random order with three levels of quality control (QC).

***Calculations***

Glucose rate of appearance (Ra), rate of disappearance (Rd) and glycerol production were calculated using standard isotope dilution equations (14) between 450-480 min and corrected for the baseline enrichment level. Glucose uptake was determined by subtracting the rate of glucose excretion from the rate of glucose disappearance.

Area under the curves (AUC) were calculated between study points 0-480 min without and with correction for the baseline (iAUC). Mean concentrations and TTRs were calculated between study points 450-480 min.

Tracer concentrations of BOHB and palmitate in NEFA and TAG pools and were calculated as APE x measured plasma concentration.

***Power calculation***

A power calculation was based on the primary endpoint, which was the plasma BOHB concentration at the end of the meal study.  It was calculated from a study which investigated the effect of 7 days treatment with SGLT2 inhibitor, luseogliflusin, on plasma BOHB, 11 h after the oral administration of the IMP, in type 2 diabetic participants in a randomized, double-blind, placebo-controlled, crossover study (15). Based on the mean differences in this study, completing the current study in 12 participants should detect a minimum mean difference in plasma BOHB concentration of 0.34 mmol/l (35.4 ng/ml) with a standard deviation of 0.38 mmol/l (39.8 ng/ml) between placebo and treatment in a cross over design with 80% power in a 2 sided t-test with 5% level of significance. The trial was terminated after 9 patients due to the COVID-19 pandemic. A retrospective power calculation showed that with 9 subjects there is 72% power to detect the observed mean difference of 0.255 in BOHB concentration (450-480) as statistically significant in a 2 sided hypothesis test with 5% level of significance. The a priori power calculation assumed a large effect size of 0.90 (=0.340/0.380), and based on a two-sided statistical test required 12 subjects to demonstrate a significant difference with 80% power. Had a one-sided test been proposed, as might have been reasonable since it has been shown previously that ketogenesis is higher with SGLT2 inhibitors, 10 subjects would have been required to demonstrate such an effect; and 9 subjects would have been sufficient to demonstrate an effect size of 0.91 or greater with the same power. The observed effect sizes were 0.99 and 0.97 respectively, so that it is unlikely that these differences were due to chance. (The post-hoc power calculations for the observed differences on the basis of a one-sided test are 85.7% and 84.0% respectively).

***Statistics***

All results are mean± SEM. The primary endpoint is the statistical evaluation of contrasts between placebo and treatment on plasma BOHB concentration, as measured at 480 minutes. Final plasma BOHB 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 subject was the random effects in the model. The denominator degrees of freedom were adjusted using Kenward-Roger approximations.

All outcome variables measured at a single point were analysed in the same way as the primary endpoint.

All outcome variables measured on repeated time points within each period, are statistically contrasted between the treatment and placebo 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, subject as random effects, time as a repeated measure with SP(POW) variance-covariance structure. Denominator degrees of freedom was adjusted using Kenward-Roger approximations.

Normality of the data was assessed using residual plots. If evidence of any non-normality was found, then the data was log 10 transformed and estimated treatment differences (and their confidence intervals) were back transformed into ratios before reporting.

Mixed model analysis was performed using SAS® version 9.4. Analysis of the acylcarnitines was by paired t-test and linear relationships were assessed by Pearson’s correlation in SPSS version 25.0.

**Results**

Nine participants completed the study (Age: 61.7 ± 3.7y, BMI: 28.9 ± 1.0 kg/m2, HbA1c: 7.7±0.2 % (60.2 ± 2.5 mmol/mol), 3F/6M). All participants were on metformin, five were taking sulphonylureas, of which four were also taking DPP4 inhibitors. Six participants were taking statins for hypercholesterolaemia and four participants were prescribed anti-hypertensives. One participant had documented ischaemic heart disease and one had known diabetic retinopathy. Duration of diabetes 8.8±0.9 y, ethnicity (8 Caucasian/1Asian). Baseline demographics are shown in Supplement Table S1 and baseline medications in Supplement Table S2.

At baseline glucagon was higher following dapagliflozin (p=0.02) but there was no difference in glucose and insulin concentrations (Table 1). Baseline BOHB, TAG and NEFA were not different but total cholesterol and LDL cholesterol were significantly lower with dapagliflozin (p=0.01, p=0.008 respectively).

With dapagliflozin, glucose AUC0-480min was lower (p=0.008). Insulin AUC0-480min (p = 0.084) and glucagon AUC0-480min (p = 0.089) tended to be different without reaching statistical significance. At 450-480min, mean glucose concentration (p=0.002) and peripheral glucose uptake (p=0.002) were lower, glucose excretion was higher (p=0.000) and glucagon concentration was higher (p=0.01) with dapagliflozin than placebo (Table 2). The significantly higher glucagon concentration with dapagliflozin was lost when the data was corrected for the baseline concentration. Endogenous glucose production was not different. There was a trend for a higher mean glucagon:insulin ratio450-480min (p=0.055). Glycerol Ra, a measure of lipolysis at 450-480min was not different between treatments.

AUCs (0-480min) for TAG concentration and NEFA concentration (Table 1) and mean concentration (450-480) for TAG palmitate TTR, NEFA palmitate concentration and NEFA palmitate TTR were not different between treatments (Table 2). Plasma NEFA concentration was higher at 300 and 420 min (p=0.04, p=0.034) but the mean NEFA concentration450-480min was not different (Figure 1, Table 2).

BOHB concentration did not change significantly in the placebo group after feeding. With dapagliflozin there was a significant effect of time (p=0.0002) and concentrations were significantly higher than placebo from 360 min onwards (Figure 1). By 450-480 min the mean difference in BOHB concentration between treatments was 0.256 ± 0.087 mmol/l (p=0.023). With both treatments BOHB TTR increased more rapidly than NEFA palmitate TTR peaking at 240 min with dapagliflozin treatment, then declining much more slowly than NEFA palmitate TTR (Figure 2). Dapagliflozin versus placebo BOHB TTR AUC0-480min (1.450±0.129 v 1.207±0.126 TTR\*min) was higher (p=0.009) and mean BOHB TTR450-480min was higher (p=0.03) (Table 2). BOHB tracer concentration AUC0-480min (0.399±0.085 v 0.154±0.030 mmol/L\*min) and mean BOHB tracer concentration450-480min were higher with dapagliflozin than placebo (p=0.019, p=0.017 respectively) (Figure 2, Table 2).

Acetyl carnitine, hydroxybutyryl carnitine, hydroxytetradecanoylcarnitine, octadecanoylcarnitine and octadecacadienylcarnitine were significantly higher with dapagliflozin (all p<0.05) (Supplement Table S3).

Baseline BOHB concentration correlated with baseline NEFA concentration with both dapagliflozin (r =0.68, p=0.04) and placebo (r=0.78, p=0.015), but there was no correlation between mean plasma concentrations of BOHB450-480min and NEFA450-480min in either treatment group. BMI negatively correlated with both BOHB450-480min concentration (r=-0.806, p=0.009) and BOHB tracer concentration450-480min ( r=-0.810, p=0.008) with dapagliflozin but not placebo.

**Conclusions**

We used a novel application of a stable isotope test to demonstrate that in type 2 diabetes dapagliflozin, compared to placebo, results in a greater increase in ketone concentration after the ingestion of olive oil. Olive oil was ingested in the absence of carbohydrate, to minimise an increase in insulin secretion, to preserve a steady state at the end of the time course. The tracer in the oil was designed to label the plasma NEFA pool with [U-13C] palmitic acid as a precursor for hepatic BOHB production to measure ketogenesis. The test was designed to initially label the plasma TAG pool and with subsequent hydrolysis by the enzyme lipoprotein lipase located on capillary endothelium, the systemic NEFA pool would become labelled (16). When part of a mixed meal, fatty acids have previously been shown to be precursors for BOHB synthesis in healthy people (17). The approach here was successful as indicated by the respective enrichment profiles. The rise in [13C2] BOHB tracer concentration with both treatments demonstrates the oxidation of the ingested [U-13C] palmitate into ketones. This was similar in both groups at the early time points but at the end of the study the higher BOHB tracer concentration with dapagliflozin suggests a higher oxidation rate to ketones. The higher concentration of both short chain and a long chain acyl carnitine with dapagliflozin is consistent with an increase in fatty acid oxidation. The accumulation of acyl carnitines has previously been documented in fasting (18) and following a liquid low-calorie diet (19), due to inefficient β-oxidation. The urinary excretion of acetylcarnitine has been shown to be correlated with blood beta-hydroxybutyrate concentration in the normal-weight subjects during fasting and in diabetic ketotic patients (20).

Glycerol production rate, a measure of lipolysis was not different between treatments. Further evidence for a lack of effect of dapagliflozin on lipolysis was the similar plasma palmitic acid TTR profile with the two treatments. An increase in lipolysis will dilute the tracer and TTR would be expected to be lower with dapagliflozin. Interestingly the rise in palmitic acid TTR was much slower than the BOHB TTR which suggests this is not the direct source of ketones at the early time points. High fat diets stimulate ketogenesis in the intestine and ketones have been measured in the portal vein. Intralipid gavage in fed mice fed a high fat diet has been shown to result in elevated ketone levels in portal blood within 30 min and in venous blood after 120 min (21). This demonstrates that ketogenesis in the intestine can increase circulating ketone levels. Thus, it is possible that some of the ingested [U-13C]palmitic acid was directly oxidised to ketones in the enterocyte. BOHB TTR was similar at the early time points in both treatments, which suggests that dapagliflozin did not increase enterocyte ketogenesis. However, although enterocyte ketogenesis may initially be the primary source, it seems unlikely that this would still be the case by 480 min. Hepatic ketogenesis from plasma palmitate is the most likely source for the rise in BOHB TTR from 120 min. The fall in plasma NEFA palmitate TTR below BOHB TTR from 450 min suggests that storage of the labelled palmitate within the liver, presumably in TAG, provides an additional source for ketogenesis.

The robust increase in the isotopic enrichment of the plasma TAG pool, which would be expected to recycle back to the liver as chylomicron remnants could also provide additional potential precursor fatty acyl moieties for BOHB synthesis. The TTR profile was similar to NEFA palmitate TTR, peaking at 180 min, albeit at a lower enrichment. This fraction is a mixture of chylomicrons and VLDL and the CM TAG TTR would be diluted by VLDL TAG.

The correlations are interesting.  Although as expected baseline BOHB concentration correlated with baseline NEFA concentration with both placebo and dapagliflozin treatment, there was no correlation between plasma BOHB and NEFA concentrations at the end of the study further suggesting plasma NEFA concentrations are not the major driver of ketogenesis. The negative relationship between BMI and BOHB concentration in the current study was also shown in our previous study of dapagliflozin in type 1 diabetes (10). This was a small group but may suggest a greater risk of BOHB and therefore ketosis in people with type2 diabetes and a lower BMI. This may be of clinical significance.

The lower glucose concentration with dapagliflozin caused by a rise in glucose excretion is well documented (1). Glucose uptake into peripheral tissues was reduced with dapagliflozin and this is the likely mechanism for the drive in ketogenesis. The brain is a major user of glucose. In the fasting state the brain accounts for at least 50% of whole body glucose uptake (22). The subjects were fasted overnight and only olive oil was ingested, so by the end of the study no carbohydrates had been ingested for 20 hours. The brain plays a central role in metabolic homeostasis, sensing glucose levels and regulating endogenous glucose production and lipolysis partly via efferent pathways to the periphery (23). β-hydroxybutyrate and acetoacetate, are the brain's main physiological alternative fuel to glucose and brain ischemia in mice induces ketogenesis in liver, mediated by B-adrenergic receptors (24). That there is higher ketogenesis with dapagliflozin compared to placebo in the absence of any difference in lipolysis and only minor changes in NEFA concentrations suggests that there is a metabolic switch in the liver to increase ketogenesis rather than this being driven by increased NEFA. This may possibly be mediated by the brain. However, participants in the current study did not develop ketoacidosis. It is likely that while there may be a metabolic switch in the liver to increase ketogenesis in response to dapagliflozin, increased rates of white adipocyte lipolysis are still necessary to develop ketoacidosis.

It is possible that the study was underpowered for NEFA concentrations. Ferrannini et al studied the effect of 28 days empagliflozin treatment in 66 patients with type 2 Diabetes in an open label study (25). Fasting mean BOHB increased from 0.3 mmol/l at baseline to 0.6 mmol/l after treatment, an increase comparable to the current study. Although fasting NEFA significantly increased (p<0.05), the increase was small, only 7.8%. However lipolysis, measured by glycerol Ra, was not different as in the current study.

We cannot exclude an effect of glucagon to increase ketosis since this was higher following dapagliflozin treatment. However, the role of glucagon in promoting ketogenesis has recently been challenged. In mice neither fasting nor SGLT2 inhibitor induced ketosis was altered by interruption of glucagon signalling (26) and in a rat model of type 2 diabetes the effect of dapagliflozin to promote ketosis was independent of hyperglucagonemia (27).

Previous studies have suggested that the rise in ketones with SGLT2 inhibitors may be due to reduced renal excretion or reduced uptake of BOHB by peripheral tissues. SGLT2 inhibition can reduce glomerular filtration rate in type 1 and 2 diabetes and reduce renal ketone excretion (28). While we cannot rule out a contribution to increased BOHB via these mechanisms, our study clearly shows dapagliflozin increases ketone synthesis.

The finding of lower cholesterol and LDL cholesterol with dapagliflozin is interesting. Most previous studies have shown dapagliflozin to either increase LDL cholesterol (29), or to have no effect in patients treated with statins (30). One study found a reduction in small dense LDL(31) while real life data reported a decrease in LDL cholesterol after 6 months treatment (32).

In summary this study shows that dapagliflozin increases the oxidation of ingested fatty acids to ketones. There is no evidence that this is driven by increased plasma NEFA concentrations as in diabetic ketoacidosis and we suggest that a metabolic switch within the liver increases ketogenesis. We also demonstrate that the initial rise in BOHB may be due to ketogenesis in the intestine.

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*Author Contributions:* R.H., D. L. R-J., A.M.U., B.F. and M.D. designed the study. M. S. and F. S-M. carried out the metabolic studies. F. S.-M. carried out sample analysis and N.J. assisted. B. M. conducted the metabolomic analysis, A. M. U., B. F. and F. S-M. interpreted the data. J. M. completed the statistical analysis. A.M.U., B.F., and F. S-M, drafted the manuscript. All authors reviewed and edited the manuscript.

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Guarantor’s statement:

Dr Roselle Herring is the guarantor of this work and as such had full access to all of the data in the study and takes responsibility for the integrity of the data of the data and the accuracy of the data analysis.

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Table 1. Baseline measurements and metabolic response (AUC0-480min) to ingestion of olive oil after 28 days treatment with Dapagliflozin or Placebo. Results are mean±SEM.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Dapagliflozin | Placebo | P-value |
| Baseline glucose (mmol/L) | 8.67±0.86 | 9.45±0.53 | 0.279 |
| Baseline BOHB (mmol/L) | 0.126±0.03 | 0.091±0.03 | 0.418 |
| Baseline NEFA (mmol/L) | 0.70±0.06 | 0.70±0.09 | 0.568 |
| Baseline NEFA palmitate (mmol/L) | 0.17±0.02 | 0.15±0.02 | 0.568 |
| Baseline TAG (mmol/L) | 1.68±0.26 | 1.77±0.20 | 0.603 |
| Baseline Total cholesterol (mmol/L) | 3.86±0.11 | 4.27±0.11 | 0.011 |
| Baseline LDL-cholesterol (mmol/L) | 1.88±0.11 | 2.23±0.13 | 0.008 |
| Baseline insulin (pmol/L) | 143±22 | 139±24 | 0.679 |
| Baseline glucagon (ng/L) | 97.9±11.5 | 83.3±9.4 | 0.024 |
| Glucose AUC0-480min mmol/L\*min | 3175±258 | 3697±212 | 0.008 |
| BOHB AUC0-480min mmol/L\*min | 116.4±17.7 | 56.6±8.9 | 0.014 |
| NEFA AUC0-480min mmol/L\*min | 357±23 | 326±18 | 0.188 |
| TAG AUC0-480min mmol/L\*min | 845±107 | 900±104 | 0.555 |
| Insulin AUC0-480min pmol/L\*min | 48346±7871 | 53614±7667 | 0.084 |
| Glucagon AUC0-480min ng/L\*min | 48091±5109 | 41884±3040 | 0.089 |
| Glucagon to insulin ratio AUC0-480min ng/pmol\*min | 598±108 | 472±84 | 0.179 |

Table 2. Mean concentration, TTR, glucose and glycerol kinetics between 450-480min after 28 days treatment with Dapagliflozin or Placebo. Results are mean±SEM

|  |  |  |  |
| --- | --- | --- | --- |
|  | Dapagliflozin | Placebo | P-values |
| Glucose450-480min (mmol/L) | 5.70±0.45 | 6.59±0.37 | 0.002 |
| BOHB450-480min (mmol/L) | 0.42±0.08 | 0.16±0.04 | 0.023 |
| NEFA450-480min (mmol/L) | 0.85±0.05 | 0.76±0.04 | 0.307 |
| NEFA palmitate450-480min (mmol/L) | 0.17±0.01 | 0.16±0.02 | 0.653 |
| Insulin450-480min (pmol/L) | 90.6±15.1 | 87.7±14.0 | 0.565 |
| Glucagon450-480min (ng/L) | 81.1±6.0 | 68.9±4.8 | 0.010 |
| Glucagon to insulin ratio450-480min (ng/pmol) | 1.17±0.27 | 0.99±0.20 | 0.055 |
| Glucose Rd450-480min (micromol/kg/min)\* | 8.50±0.55 | 10.98±0.72 | 0.002 |
| Glucose Ra450-480min (micromol/kg/min) | 9.84±0.85 | 10.24±0.74 | 0.542 |
| Urinary glucose excretion450-480min (micromol/kg/min) | 3.019±0.344 | 0.009±0.002 | 0.000 |
| Glycerol Ra450-480min (micromol/kg/min) | 2.90±0.21 | 2.94±0.35 | 0.878 |
| BOHB TTR450-480min x10-3 | 3.00±0.46 | 1.99±0.39 | 0.030 |
| BOHB tracer conc450-480min mmol/Lx10-3 | 1.50±0.56 | 0.40±0.14 | 0.017 |
| NEFA palmitate TTR450-480min x10-3 | 1.96±0.22 | 2.25±0.29 | 0.368 |
| NEFA palmitate tracer conc450-480min mmol/Lx10-3 | 0.32±0.04 | 0.32±0.05 | 0.906 |
| TAG TTR450-480min x10-3 | 2.58±0.15 | 2.73±0.15 | 0.476 |
| TAG tracer conc450-480min mmol/Lx10-3 | 1.79±0.35 | 2.15±0.56 | 0.788 |

\* Calculated as Rd-Glu excretion

Diagram

Description automatically generated Fig 1. Metabolite and hormone concentrations following ingestion of olive oil after 28 days treatment with Dapagliflozin or Placebo. Results are mean±SEM. a) Glucose, b) NEFA, c) BOHB, d) TAG, e) Insulin, f) Glucagon. \* time point differences between treatments p<0.05. Black circles dapagliflozin, white circles placebo.

Diagram

Description automatically generated

Fig 2. BOHB and palmitate TTR and tracer concentration following ingestion of olive oil after 28 days treatment with Dapagliflozin or Placebo. Results are mean±SEM.

a) NEFA palmitate TTR, b) NEFA palmitate tracer concentration, c) BOHB TTR d) BOHB tracer concentration e) TAG palmitate TTR f) TAG palmitate tracer concentration. \* time point differences between treatments p<0.05. Black circles dapagliflozin, white circles placebo.