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ORIGINAL ARTICLE

Sustained fasting glucose oxidation and postprandial lipid oxidation associated with reduced insulin dose in type 2 diabetes with sodium–glucose cotransporter 2 inhibitor: A randomized, open-label, prospective study

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ABSTRACT

Aims/Introduction: Hyperglycemia impairs energy substrate oxidation as a result of glucotoxicity. We examined whether the reduction of plasma glucose using a sodium–glucose cotransporter 2 inhibitor, in inpatient diabetes management, has any effect on: (i) treatment period and basal–bolus dosage of insulin that achieve euglycemia; (ii) fasting/postprandial energy expenditure (EE); and (iii) energy substrate oxidation.

Materials and Methods: This was a randomized, open-label, 7-day prospective study. Participants were type 2 diabetes patients with hyperglycemia, aged >20 years, with glycated hemoglobin >10%, daily mean preprandial blood glucose >11 mmol/L (200 mg/dL) and no previous antidiabetic medication. A total of 18 type 2 diabetes patients were randomized (1:1) to basal–bolus insulin titration algorithm (INS) alone or INS + dapagliflozin 5 mg/day (INS/DAPA). The main outcome measures were total daily insulin dose to achieve euglycemia, as well as EE and respiratory quotient during fasting and postprandial states, measured by indirect calorimetry.

Results: The rate of euglycemia was higher in the INS/DAPA compared with INS group (100 vs 55.6%, $P = 0.04$), whereas the total daily dose of insulin was 19% lower and was accompanied by a decreased basal–bolus ratio ($P = 0.02$). Fasting and postprandial EE elevation were similar in both groups. The post-treatment fasting respiratory quotient significantly increased in the INS/DAPA group (0.72 ± 0.05 vs 0.79 ± 0.08, $P = 0.04$), and the postprandial respiratory quotient elevation was abolished; the opposite trend was observed in the INS group ($P < 0.02$).

Conclusions: INS/DAPA sustained fasting carbohydrate oxidation, postprandial lipid-derived EE (failed to increase carbohydrate-derived EE) and reduced basal insulin requirement might be related to further bodyweight loss.

Clinical Trial Registry: *National University Hospital Medical Information Network* UMIN000018997

INTRODUCTION

Type 1 and type 2 diabetes are chronic debilitating diseases that are associated with excessive morbidity and premature mortality[1,2]. The major clinical manifestation of all types of diabetes is an elevated blood glucose concentration. Diabetes and its complications are caused by chronic glucotoxicity driven by persistent hyperglycemia. The incidences of diabetes-related complications have decreased in the past two decades, most

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likely because of the increasing awareness and improved management of diabetes[3]. Nevertheless, diabetes and its complications continue to place significant burdens on healthcare systems.

Although many pathways are activated or upregulated to remove excess glucose, persistent hyperglycemia and glucotoxicity lead to the deterioration of cellular and/or organ function[4].

Drugs that lower blood glucose concentrations in patients with type 1 and 2 diabetes are intended to reduce the symptoms of diabetes and the risk of long-term complications. A recently-introduced class of drugs, sodium–glucose cotransporter 2 (SGLT2) inhibitors, has been shown to improve diabetic complications and reduce all-cause mortality[5,6]. Inhibition of SGLT2 in the proximal convoluted tubule suppresses sodium and glucose reabsorption, and promotes their elimination in urine, and hence improves glycemic control and ultimately attenuates the progression of diabetes-related complications[5,7].

Increasing insulin dosage by means of overcoming hyperglycemia is the mainstay to resolve insulin resistance as a result of glucose toxicity. Despite extracellular hyperinsulinemia using insulin injections, a blunted intracellular glucose oxidation pathway might delay the time to improve glucose oxidation metabolism. To address achieving euglycemia, instead of oversupplying the excess glucose to further incorporate into the cells with insulin, we thought eliminating glucose to urine would facilitate the time to euglycemia. Therefore, we aimed to determine whether short-term urinary elimination of glucose could reduce a time to achieving euglycemia, and the insulin requirement in patients receiving intensive insulin treatment. We also aimed to assess macronutrient oxidation, the capacity to switch fuel oxidation to adjust for fuel availability (i.e., metabolic flexibility) and to probe the mechanisms responsible for glucose elimination to urine. Furthermore, we evaluated the impact of urinary elimination of glucose, on the selective substrate energy utilization assessed by indirect calorimetry, in the setting of fasting and postprandial state.

SGLT2 inhibitors have proven efficacy in type 2 diabetes, and several members of this class have been approved for clinical use. SGLT2 inhibitors have also been proposed for the management of type 1 diabetes[8,9], and several clinical trials have explored the efficacy and safety of SGLT2 inhibitors in this setting[10–12]. The use of oral hypoglycemic agents, such as SGLT2 inhibitors, is not without risk; indeed, their use might be limited in insulin-treated patients with type 1 diabetes because of the increased risk of euglycemic ketoacidosis in patients lacking intrinsic insulin secretion[13]. Although type 2 diabetes is predominantly characterized by insulin resistance, its progression might lead to insulin deficiency requiring insulin administration. Thus, it is important to examine the insulin doses required to achieve euglycemia in insulin-treated type 2 diabetes patients with SGLT2 inhibitor, subsequently, considering the impact of substrate energy utilization (i.e., enhanced elimination of carbohydrate via urine) on energy expenditure (EE) and respiratory quotient (RQ).

From this context, the primary objective of the present study was to gain further insight into the insulin requirement and time to resolve euglycemia, combination with EE, and metabolic fuel selectivity associated with SGLT2 inhibition in patients with non-insulin-dependent type 2 diabetes and uncontrolled hyperglycemia. Thus, patients with severe hyperglycemia might benefit from the reduction in acute cellular stress achieved through the elimination of excess extracellular glucose to urine induced by SGLT2 inhibitors. We hypothesized that adding an SGLT2 inhibitor to insulin treatment could reduce the dose of insulin and the period to achieve euglycemia, and subsequently to reduce the metabolic EE compared with administration of insulin alone. We also hypothesized that during energy loss, adaptive thermogenesis occurs where EE is suppressed beyond that predicted for the calorie loss in urine.

METHODS

Study design and ethics

This was a 1-week, open-label, randomized (1:1), prospective trial carried out in an inpatient setting at the Faculty of Medicine, Toho University School of Medicine, Tokyo, Japan, to evaluate the effects of co-administration of an SGLT2 inhibitor with insulin on the metabolic status of patients with type 2 diabetes requiring hospitalization for the management of severe hyperglycemia. It was carried out between October 2014 and November 2015. The protocol was reviewed by the Japanese authority in accordance with local regulations, and was reviewed and approved by the institutional review board of Toho University Omori Medical Center (No. 27-132). The study was carried out in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. The study was registered on the National University Hospital Medical Information Network (UMIN Clinical Trials Registry: UMIN000018997), and the trial protocol is available in Figure S1 and Appendix S2.

Participants

Patients with type 2 diabetes requiring hospitalization to control hyperglycemia were eligible if they were aged >20 years, had a hemoglobin A1c (HbA1c of >10%) and daily mean preprandial blood glucose >11 mmol/L (200 mg/dL). Patients who had been administered any previous antidiabetic medication (including insulin) were excluded. Patients with any of the following were also excluded: type 1 diabetes or secondary diabetes caused by another condition; myocardial infarction <3 months before enrollment or known heart failure; history of hypersensitivity to the study drugs; history of diabetic ketoacidosis or diabetic coma, or at risk of diabetic coma; severe liver disease; severe renal disease; severe pancreatic disease; hemoglobin (Hb) <11 g/dL; current malignancy; platelet count <100,000/mm^3; severe diabetic neuropathy; proliferative retinopathy; serious infection; recent surgery or severe trauma;

or excessive alcohol consumption. Pregnant women or possibly pregnant women were also excluded. Patients judged unsuitable for the study by the attending physician were also excluded. Written informed consent was obtained from all participants.

Interventions

A total of 20 patients with type 2 diabetes were randomized (1:1) to initiate a basal–bolus insulin titration algorithm (insulin group) or dapagliflozin at a dose of 5 mg/day in combination with the insulin regimen (insulin + dapagliflozin group) on admission. The randomization scheme was generated by an independent researcher (using a random number generator) who was not directly involved in data collection or delivery of the study drugs. Both groups received the same insulin regimen. Randomization was carried out using the minimization method with the following background factors: blood glucose level and age. The target fasting and postprandial blood glucose measures were 4.5–6.1 mmol/L (80–110 mg/dL) and 4.5–7.8 mmol/L (80–140 mg/dL), respectively. The insulin dose was adjusted using an insulin algorithm, which was adapted from a previous study[14], according to the blood glucose concentration measured the previous day using a glucose meter (One Touch; Johnson & Johnson, New Brunswick, NJ, USA). The starting dose of insulin was 0.2 IU/kg/day (1:1 basal–bolus). For fasting, blood glucose concentrations of 6.1–7.7 mmol/L (110–139 mg/dL), 7.8–9.9 mmol/L (140–179 mg/dL) and >10 mmol/L (>180 mg/dL), the basal insulin dose (insulin glargine) was increased by 2, 3 and 4 IU, respectively. For postprandial, blood glucose concentrations of 7.8–9.9 mmol/L (140–179 mg/dL) and >10 mmol/L (>180 mg/dL), the bolus insulin dose (insulin aspart) was increased by 2 and 3 IU, respectively.

Procedures and study end-points

Patients received meals three times per day (08.00, 12.00, 18.00 hours) in an inpatient setting. For patients with a body mass index (BMI) >25 kg/m^2, the total daily calorie content was 25 kcal/kg, divided evenly among breakfast, lunch and dinner. For patients with a BMI ≤25 kg/m^2, the total daily calorie content was 27 kcal/kg.

Indirect calorimetry (CARESCOPE Monitor B650®; GE Healthcare, Hino, Japan) was carried out in the same environment on pre-treatment day and post-treatment day 7. After an overnight fast and a 1-h basal period, patients consumed a mixed meal (within 10 min) comprising 60% carbohydrate, 25% fat and 15% protein with a total calorie content of 25 kcal/kg for patients with a BMI ≥25 kg/m^2, or 27 kcal/kg for patients with a BMI <25 kg/m^2. Indirect calorimetry was carried out for 10-min periods at the following times: −30 to −20 min before the meal, and at 30–40 min and 90–100 min after the meal. Blood samples were obtained at the same time points.

The primary efficacy end-point was the total daily insulin dose required to achieve euglycemia, which was defined as a daily mean preprandial blood glucose of ≤7.8 mmol/L (140 mg/dL). The area under the curve of the mean (7-day) preprandial blood glucose concentrations in the experimental period was calculated using the trapezoidal method.

Secondary efficacy end-points included EE and RQ, as measured by indirect calorimetry carried out on pre-treatment day and post-treatment day 7. Oxygen consumption and carbon dioxide production were measured to calculate EE and RQ. The substrate utilization indicator for protein oxidation (urine urea nitrogen) was used to assess nitrogen balance. EE and RQ were assessed at steady state, which was defined as the 5-min period of time in which the average minute-by-minute changes in oxygen consumption and carbon dioxide production were <10% and the average RQ change was <5%[15,16]. The average RQ and EE at steady state were measured in each 10-min recording period.

Exploratory end-points included the changes in daily urinary glucose excretion, 3β-hydroxybutyrate and acetoacetate concentrations, bodyweight, systolic blood pressure, and diastolic blood pressure.

Safety was assessed in terms of adverse events, hypoglycemic events and laboratory test values. Adverse events were evaluated by the attending physicians. The avoidance of diabetic ketoacidosis was evaluated by measuring plasma and urine ketone bodies, with self-monitoring of 3β-hydroxybutyrate four times/day (fasting, before lunch, before dinner and before bedtime).

Statistical analysis

The sample size was calculated based on a mean difference in the change in fasting blood glucose between the two groups of 0.78 mmol/L (14 mg/dL) and a standard deviation of 0.15 mmol/L (2.7 mg/dL), considering the results of two prior studies of dapagliflozin[17,18]. At a significance level of 5% and power of 80%, 18 patients were required (9 per group). Therefore, we planned to enroll 10 patients per group to account for potential dropouts.

Efficacy data were analyzed in the full analysis set, defined as all patients who were registered in the study, randomized to either group, who received at least one dose of the study drug, and in whom some data were recorded after the start of treatment. Safety analyses were carried out in all registered patients and those who received at least one dose of the allocated study treatment.

The results are presented as the mean ± standard deviation or the median (interquartile range) for normally and non-normally distributed variables, respectively. The within-group changes in variables were analyzed using paired *t*-tests or Wilcoxon signed-rank tests for normally and non-normally distributed variables, respectively. Analysis of covariance was used to compare the changes in efficacy variables over time between the two groups. *P*-values of <0.05 were considered statistically significant. All analyses were carried out using JMP 12 (www.JMP.com).

RESULTS

Patient characteristics

A total of 20 patients were initially screened for this study on admission. Among these, two patients withdrew their consent, and the remaining 18 were randomly assigned to insulin + dapagliflozin or insulin alone (Figure 1). The baseline characteristics of patients in both groups were similar (Table 1). In the total study population, the mean ± standard deviation age was 50.7 ± 11.9 years, BMI was 26.8 ± 4.9 kg/m^2, diabetes duration was 2.1 ± 2.8 years, HbA1c was 13.0% ± 1.4% and fasting plasma glucose concentration was 16.5 ± 5.1 mmol/L (297 ± 92 mg/dL). The serum ketone body concentration before starting treatment was 1,056 ± 2,365 μmol/L, and was similar in both groups. The

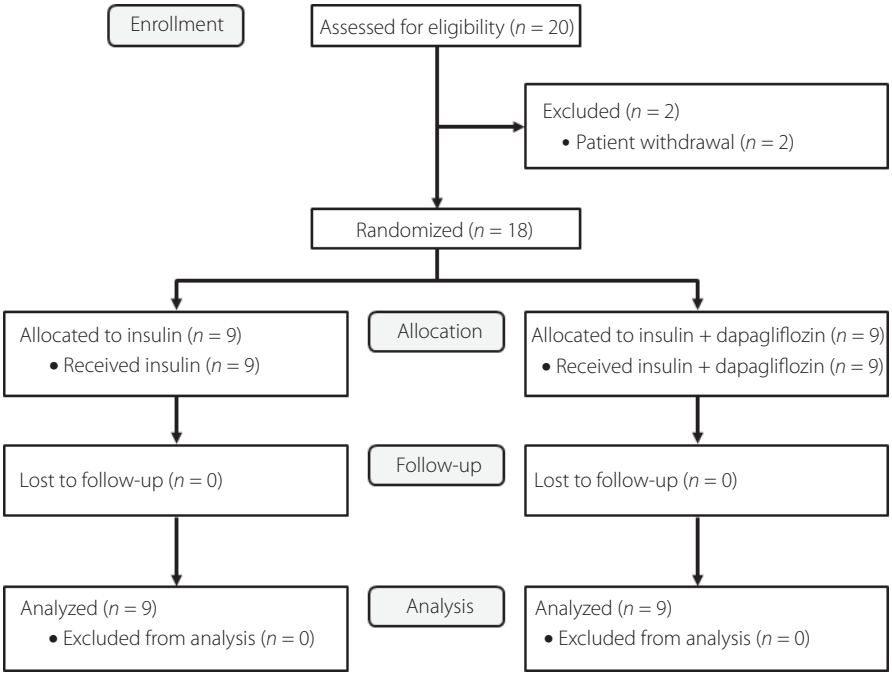

Figure 1 | Patient disposition.

Table 1 | Patient characteristics

	All patients	Insulin	Insulin + dapagliflozin	*P*-value
n (male/female)	18 (15/3)	9 (7/2)	9 (8/1)	
Age (years)	50.7 ± 11.9	55 ± 13.3	46.3 ± 9.02	0.184
Diabetes duration (years)	2.1 ± 2.8	2 ± 2.64	2.2 ± 3.3	0.439
Bodyweight (kg)	76.1 ± 14.9	73 ± 13.6	79.1 ± 16.3	0.59
BMI (kg/m^2)	26.8 ± 4.9	26.4 ± 4.76	27.3 ± 5.3	0.95
HbA1c, NGSP (%)	13 ± 1.4	13 ± 1.33	12.9 ± 1.48	0.93
FBG (mmol/L)	16.5 ± 5.1	16.8 ± 6.8	16.2 ± 2.9	0.72
Fasting insulin (μmol/mL)	9 ± 10	5.82 ± 2.8	12.2 ± 13	0.36
Fasting C-peptide (ng/mL)	2.25 ± 1.2	1.8 ± 0.58	2.5 ± 1.5	0.32
eGFR (mL/min/1.73 m^2)	95.7 ± 15.5	100.27 ± 15	90.1 ± 17.5	0.07
Total cholesterol (mg/dL)	234.5 ± 51.1	227.3 ± 60.2	239.6 ± 34.7	0.25
LDL cholesterol (mg/dL)	143.4 ± 36.4	127 ± 37.4	160 ± 28.4	0.14
HDL cholesterol (mg/dL)	42.9 ± 12.6	42.2 ± 13.8	43.6 ± 10.3	0.63
Triglycerides (mg/dL)	240.6 ± 257.8	290.3 ± 324.3	190.9 ± 118.9	0.41

Values are expressed as the mean ± standard deviation. BMI, body mass index; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NGSP, National Glycohemoglobin Standardization Program.

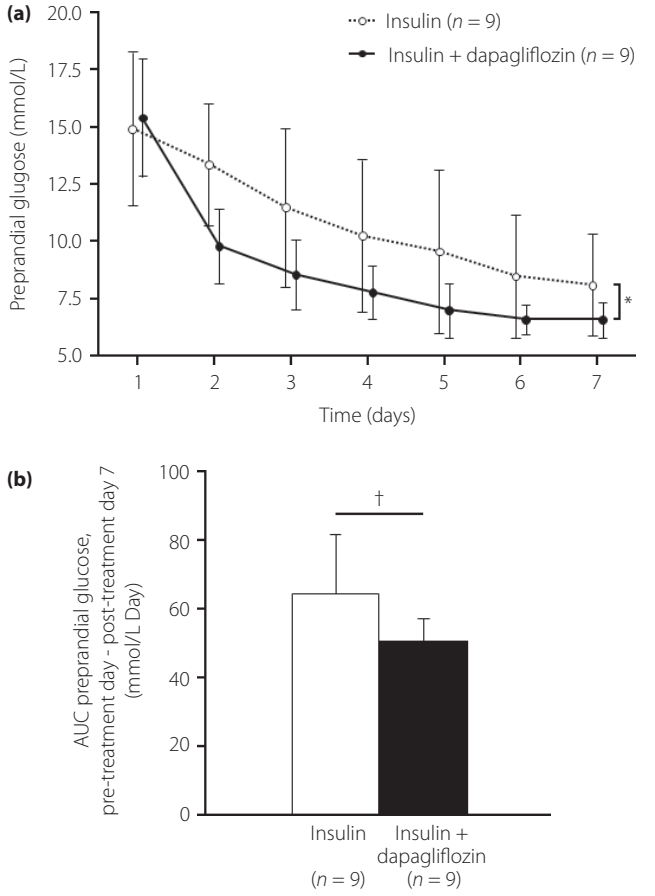

Figure 2 | (a) Daily mean preprandial blood glucose concentrations and (b) area under the curve (AUC) of daily mean preprandial blood glucose concentrations (insulin group, $n = 9$; insulin + dapagliflozin group, $n = 9$). *$P < 0.05$ (Wilcoxon signed-rank test); †$P < 0.05$ (Student's *t*-test).

reference range for the serum ketone body concentration in our institution was <130 μmol/L.

Efficacy

The proportion of patients who achieved euglycemia was significantly greater in the insulin + dapagliflozin group (9/9 patients [100%]) than that in the insulin group (5/9 patients [55.6%]; $P = 0.04$). The area under the curve of mean preprandial blood glucose from baseline was significantly lower in the insulin + dapagliflozin group than that in the insulin group (50.7 ± 6.5 vs 64.6 ± 17.2 mmol/L/day [912.6 ± 116.8 vs 1,163.5 ± 310.3 mg/dL/day], $P < 0.05$; Figure 2). Co-administration of dapagliflozin was associated with significantly lower insulin doses between post-treatment days 5–7 in terms of the total insulin dose (1.48 ± 0.32 vs 1.81 ± 0.38 U/kg, $P < 0.05$) and the basal/bolus ratio (0.41 ± 0.96 vs 0.47 ± 0.77, $P < 0.05$) compared with the insulin group (Figure 3). Urinary glucose excretion was high in both groups at baseline, as might be expected considering the severity of hyperglycemia (Table 2). Urinary glucose excretion declined on post-treatment day 1 in the insulin group after starting insulin therapy, but was maintained in the insulin + dapagliflozin group (Table 2). However, urinary glucose excretion was reduced at post-treatment day 7 in both groups relative to baseline. The serum 3β-hydroxybutyrate and acetoacetate concentrations tended to decrease in the insulin group during the study period, but remained significantly greater in the insulin + dapagliflozin group compared with the insulin group at post-treatment day 7. However, as shown in Figure 4a,b, there were no marked differences in total EE in either group in fasting or postprandial conditions, even after the achievement of euglycemia and increased calorie loss as glucosuria. In both groups, the total EEs were greater at 30 and 90 min after the meal than in fasting conditions ($P < 0.05$), consistent with the thermic effect of calorie

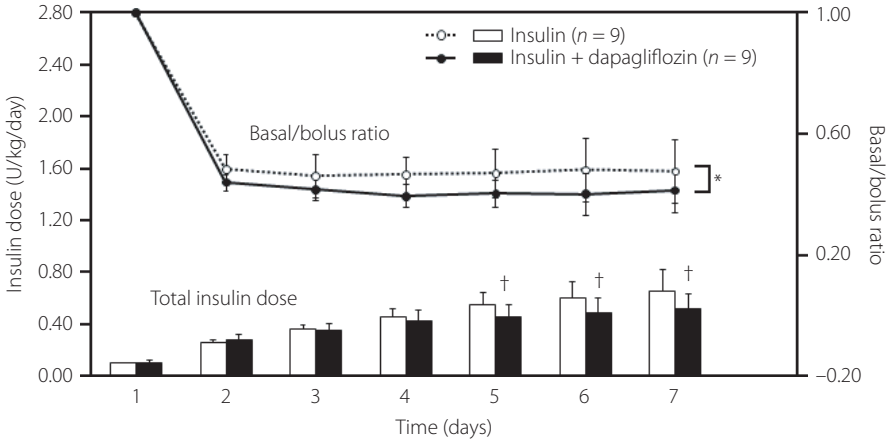

Figure 3 | Total insulin dose (lines) and the basal/bolus insulin ratio (bars; insulin group, $n = 9$; insulin + dapagliflozin group, $n = 9$). *$P < 0.05$ (Wilcoxon signed-rank test) †$P < 0.05$ (Student's *t*-test).

intake. The mean difference in thermic effect of the meal was similar in both groups. The RQ at 30 and 90 min after the meal was similar before and after treatment in the insulin and the insulin + dapagliflozin groups (Figure 4c,d). After treatment, the fasting RQ was significantly greater in the insulin + dapagliflozin group compared with that in the insulin group (0.78 ± 0.07 vs 0.72 ± 0.05, $P < 0.05$). There were no significant changes in bodyweight, systolic blood pressure or diastolic blood pressure in either group, and these variables were not significantly different between the two groups (Table 2).

Safety

Treatment-emergent adverse events were observed in one patient (11.1%) in the insulin alone group (nausea), and in one patient (11.1%) in the insulin + dapagliflozin group (nausea and decreased appetite in the same patient).

DISCUSSION

In the present 1-week study, the proportion of patients who achieved euglycemia was higher (100 vs 55.6%, $P = 0.04$), but the total insulin daily dose was 19% lower and was accompanied by a decrease in the basal/bolus ratio in the insulin + dapagliflozin group compared with the insulin group. These results indicate that, at least in the short term, treatment with an SGLT2 inhibitor in combination with intensified insulin therapy might improve glycemic control in concert with a lower insulin dose in patients who require hospitalization for glycemic management. The lower insulin doses in the insulin + dapagliflozin group are likely to be related to the increase in urinary glucose excretion. Of note, the urinary glucose excretion rate in this group was elevated from post-treatment day 3 to the end of the study (post-treatment day 7; data not shown).

Hyperglycemia is known to impair energy substrate oxidation in fasting[19] and postprandial[20,21] states, through impaired utilization of the intracellular energy source. Insofar as increased elimination of glucose into the urine is expected to reduce glucose availability, which can be used as an intracellular energy source. Therefore, we examined the effects of co-administration of an SGLT2 inhibitor with intensified insulin therapy for the treatment of severe hyperglycemia, and whether this combination was capable of resolving glucotoxicity and achieving euglycemia within 1 week. The length of the study was in line with that used by Roberts *et al.*[22], who assessed the efficacy of basal/bolus insulin versus sliding-scale insulin in a tertiary hospital setting over an 8-day period.

Thus, adding an SGLT2 inhibitor to intensive insulin therapy might have some properties to overcome glucotoxicity[8,15,23–25] by reducing glucose availability and lowering the doses of insulin required to achieve glycemic control.

Co-administration of insulin and dapagliflozin increased urinary glucose excretion as anticipated, but also increased serum

Table 2 | Changes in efficacy and safety variables during the study

Study period	All ($n = 18$)	Insulin ($n = 9$)		Insulin + dapagliflozin ($n = 9$)	
	Pre-treatment day	Post- treatment day 1	Post-treatment day 7	Post-treatment day 1	Post-treatment day 7
Bodyweight (kg)	70.9 ± 10.9	73.2 ± 13.6	73.0 ± 13.9	81.5 ± 13.6	78.7 ± 15.9
SBP (mmHg)	117.4 ± 14.9	108.9 ± 13.2	112 ± 16.9	125.9 ± 10.9	114.3 ± 11.8
DBP (mmHg)	73.3 ± 9.3	69.6 ± 5.8	61.6 ± 4.8	77.1 ± 10.6	72.9 ± 9.8
Mean preprandial plasma glucose (mg/dL)	276 ± 55.6	268.5 ± 48	145.9 ± 41†,‡	277.2 ± 46.1	118.3 ± 13.8†
Total insulin dose (U/kg/day)	0	0.1 ± 0.01	0.66 ± 0.16‡	0.11 ± 0.02	0.52 ± 0.12
Basal insulin dose (U/kg/day)	0	0.05 ± 0.02	0.21 ± 0.06‡	0.05 ± 0.01	0.15 ± 0.06
Basal/bolus insulin ratio	0	1	0.48 ± 0.11§	1	0.41 ± 0.15
Mean urine output (L/day)	2.1 ± 2.2	1.9 ± 0.68	1.5 ± 0.56‡	2.8 ± 1.5	2.5 ± 1.1
Mean urine glucose excretion (g/day)	78.5 ± 70.8	42.1 ± 28.7	0.6 ± 0.8†,‡	85.8 ± 83.1	41.2 ± 28.4§
Mean urine energy loss (kcal/day)	321.9 ± 290.3	172.6 ± 117.7	2.46 ± 3.3†,‡	351.8 ± 340.7	168.9 ± 116.4
Nitrogen balance (g/day)	76.8 ± 33.1	59.5 ± 27.5†	37.2 ± 25.9†	94.2 ± 38.7	58.3 ± 25.7
Fasting total ketone body (μmol/L)	1223.3 ± 2405.0	1191.4 ± 2126.5	199.9 ± 153.9‡	938.4 ± 1294.6	523.6 ± 268.6
Fasting 3-hydroxybutyrate (μmol/L)	828.4 ± 1553.2	521.4 ± 657.4	136.3 ± 103.5‡	733.9 ± 1068.7	445 ± 230.4
Fasting lactate (mmol/L)	18.8 ± 3.5	15.5 ± 1.6	17.6 ± 4.2	16.6 ± 6.5	15.6 ± 3.2

Values are expressed as the mean ± standard deviation. $^{\dagger}P \leq 0.05$ versus respective baseline value by paired *t*-test or Wilcoxon signed-rank test. $^{\ddagger}P \leq 0.05$ vs insulin + dapagliflozin by unpaired *t*-test or Mann–Whitney test. $^{\S}P \leq 0.05$ versus insulin + dapagliflozin by ANOVA. DBP, diastolic blood pressure; SBP, systolic blood pressure.

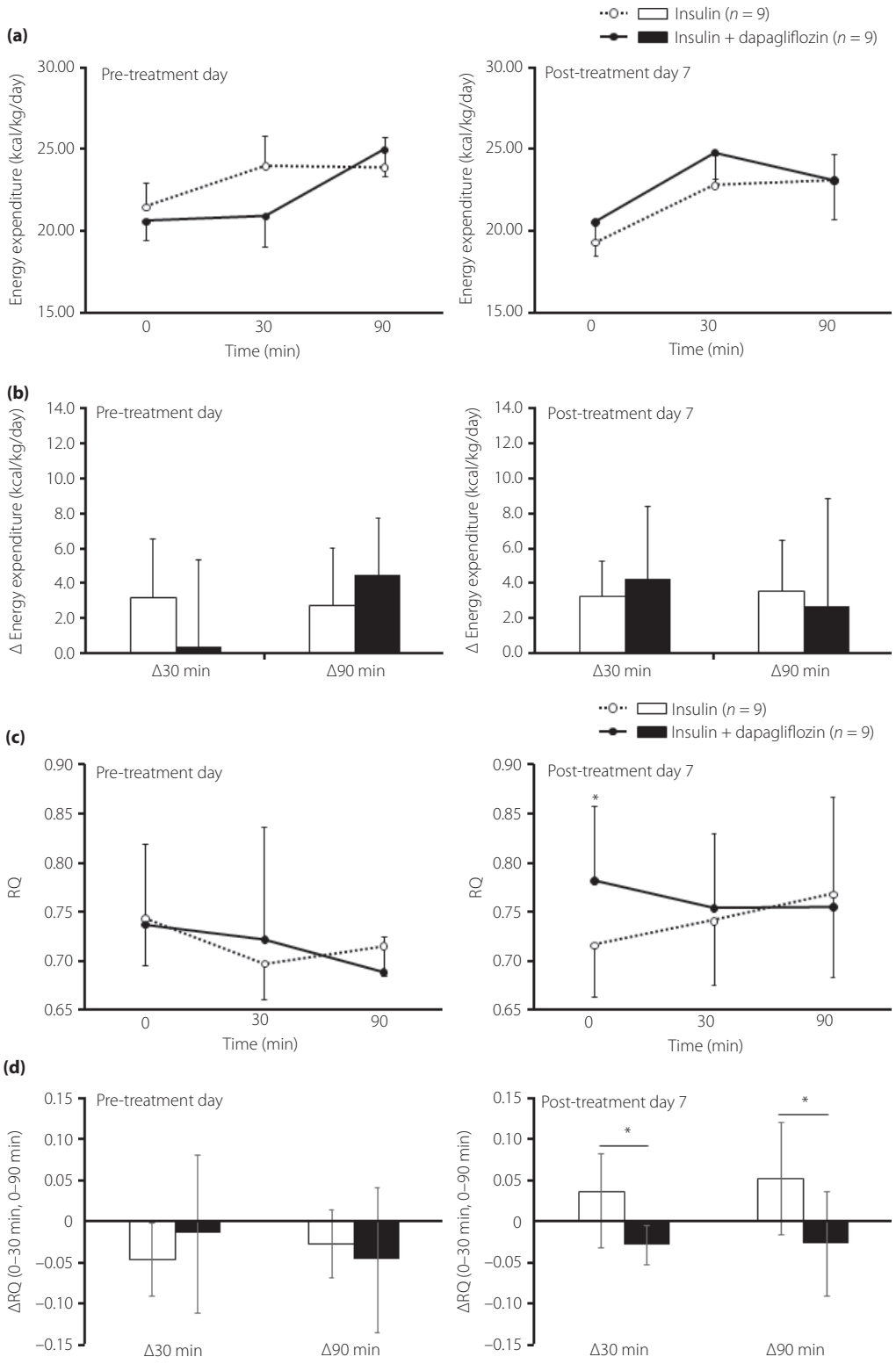

Figure 4 | (a,b) Energy expenditure and (c,d) respiratory quotient (RQ) on pre-treatment day and post-treatment day 7. (a) Energy expenditure and (c) RQ were measured in fasting and postprandial conditions after a mixed meal. Changes in (b) total energy expenditure and (d) RQ were measured at 30 and 90 min after the meal and are shown relative to 0 min (insulin group, $n = 9$; insulin + dapagliflozin group, $n = 9$). *$P < 0.05$ (Student's *t*-test).

ketone body formation regardless of the improvement in glycemic control. Intriguingly, the serum lactate concentration hardly changed during the study and was similar in both groups. These findings suggest that the risks of increased anaerobic glycolysis and pseudohypoxia attributable to oxidative stress are negligible. Thus, administration of insulin together with an SGLT2 inhibitor implicates increased fasting carbohydrate oxidation and sustained fatty acid oxidation in the postprandial state, rather than available carbohydrate after the meal, in patients with severe hyperglycemia and poor endogenous insulin secretion. In insulin-dependent patients with type 1 diabetes, administration of an SGLT2 inhibitor was reported to increase circulating ketone body concentrations and increase the risk of ketoacidosis[13,26]. Thus, monitoring ketone body concentrations and administration of an adequate insulin dose are necessary to minimize the risk of ketoacidosis. We measured serum ketone bodies and 3-hydroxybutyrate concentrations to detect possible signs of ketoacidosis in terms of safety management for poorly controlled diabetes.

SGLT2 inhibitors enhance urinary glucose excretion, and hence increase calorie loss[27]. Resting EE and meal-induced thermogenesis were similar in both groups in the present study. We first hypothesized that during urinary energy loss (i.e., glucosuria), the difference between insulin and insulin + dapagliflozin group was ~165 kcal/day, adaptive thermogenesis occurs where EE was suppressed beyond that predicted for the calorie loss in urine. However, energy consumption did not differ between treatment groups, and this indicates that basal EE and diet-induced thermogenesis were more likely to respond to ingested calories rather than the whole-body net calorie balance, as the insulin + dapagliflozin group maintained lower calories by losing extra urinary glucose (=calories). Indeed, SGLT2 inhibitors are associated with reductions in bodyweight, which appears to be mediated by increased calorie loss despite the lack of change in whole-body EE. The mean urinary glucose excretion rate was 41.2 g/day on post-treatment day 7 in the insulin + dapagliflozin group, approximately 9% of the total daily energy intake.

Large shifting between fasting and postprandial RQ was defined as metabolic flexibility in healthy, physically active individuals. Conversely, obesity, diabetes and individuals with sedentary behavior were associated with decreased RQ variation (i.e., metabolic inflexibility) in terms of poor fasting/postprandial RQ difference. All of the type 2 diabetes patients in the study had poor glycemic control with high HbA1c (mean HbA1c 13.0 ± 1.4%), potentially indicating that RQ variation between fasting and postprandial RQ was negligibly small. The fasting RQ, which indicates the preference for resting metabolic substrate oxidation, was significantly greater in the insulin + dapagliflozin group than in the insulin group. However, a previous rat model of early type 2 diabetes showed that SGLT2 inhibitor reduces RQ. In the present study, the participants were well past early diabetes and lacked metabolic control, which might have contributed to the higher fasting RQ that was different from the animal study[20]. Furthermore, the postprandial RQ elevation (postprandial 30 and 90 min, compared with the fasting), which represents postprandial metabolic flexibility, was abolished in the insulin + dapagliflozin group relative to the elevation observed in the insulin group. These results might suggest that lipids were preferentially used as the energy source in the insulin + dapagliflozin group in the postprandial state, despite carbohydrate availability. The two major features of reduced metabolic flexibility seen in the insulin + dapagliflozin group were decreased fat oxidation in the fasting state (i.e., higher RQ) and decreased insulin-stimulated carbohydrate oxidation. Impaired insulin-stimulated glucose uptake in skeletal muscle[28], impaired skeletal muscle mitochondrial biogenesis and decreased capacity for oxidation of dietary fat contribute to reduced metabolic flexibility[29]. Therefore, the present results show that co-administration of an SGLT2 inhibitor with intensive insulin therapy might be associated with differences in metabolic fuel selection in the fasting and postprandial states in patients with poorly controlled type 2 diabetes.

There were several limitations to the present study. First, we only enrolled inpatients with type 2 diabetes; however, this was necessary, because we needed to regularly obtain blood and urine samples to measure metabolic substrates, and measure EE and RQ at multiple times under controlled conditions.

Insofar, the results were obtained from a single meal per day for 1 week, and cannot be generalized to an array of type 2 diabetes patients. Second, we could not evaluate the effects of ketone bodies or the activities of different glycolytic pathways using tracer methodologies. Finally, the short treatment period (7 days) might be too short to observe clinically meaningful changes in some variables; therefore, long-term studies are necessary to verify the present results.

In conclusion, administration of an SGLT2 inhibitor at the time of starting intensive insulin therapy in patients with poorly controlled type 2 diabetes significantly reduced the insulin doses required and increased the proportion of patients achieving euglycemia within a short period of time. These results were associated with an increase in RQ in the fasting state and enhanced fatty acid oxidation in the postprandial state. Renal elimination of glucose might enhance bodyweight loss by promoting calorie loss in urine with sustained EE, resulting in further negative net energy balance.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Study protocol.
Appendix S1 | CONSORT checklist.