Effect of Dapagliflozin With and Without Acipimox on Insulin Sensitivity and Insulin Secretion in T2DM Males

[1]Aurora Merovci*, [1]Muhammad Abdul-Ghani*, [2]Andrea Mari, [1]Carolina Solis Herrera, [1]Juan Xiong, [1]Giuseppe Daniele, [1]Devjit Tripathy, [1]Ralph DeFronzo

[1]Division of Diabetes, University of Texas Health Science Center at San Antonio, and [2]Institute of Biomedical Engineering, National Research Council, Padova, Italy.

Aim: To investigate the effect of lowering the plasma glucose and free fatty acid concentrations with dapagliflozin and acipimox, respectively, on insulin sensitivity and insulin secretion in T2DM individuals.

Methods: 14 male T2DM patients received an OGTT and euglycemic hyperinsulinemic clamp at baseline and were treated for three weeks with dapagliflozin (10 mg per day). During week 3, acipimox (250 mg four times per day) treatment was added to dapagliflozin. The OGTT and insulin clamp were repeated at the end of weeks 2 and 3.

Results: Dapagliflozin cause glucosuria and significantly lowered the plasma glucose concentration (by 35 mg/dl, $P<0.01$) while the fasting plasma FFA concentration was unaffected. Acipimox caused a further decrease in the fasting plasma glucose concentration (by 20 mg/dl, $P<0.01$) and a significant decrease in the fasting plasma FFA concentration. Compared to baseline, insulin-mediated glucose disposal increased significantly at week 2 (from 4.48 ± 0.50 to 5.30 ± 0.50 mg/kgmin, $p<0.05$). However, insulin-mediated glucose disposal at week 3 (after the addition of acipimox) did not differ significantly from that at week 2. Glucose-stimulated insulin secretion at week 2 increased significantly compared to baseline and it increased further and significantly at week 3 compared to week 2.

Conclusion: Lowering the plasma glucose concentration with dapagliflozin improves both insulin sensitivity and beta cell function, while lowering plasma FFA concentration by addition of acipimox to dapagliflozin improves beta cell function without significantly affecting insulin sensitivity.

Beta cell dysfunction and insulin resistance are the core pathophysiologic defects responsible for the development of type 2 diabetes mellitus (T2DM) (1). The etiology of both insulin resistance and beta cell dysfunction is complex and involves genetic and environmental factors (2). Although genetic background contributes to the development of both insulin resistance and beta cell dysfunction, environmental factors also play an important role in the development of both conditions (2). It is well established that increased plasma free fatty acid concentration and ectopic lipid deposition play a central role in the pathogenesis of insulin resistance and beta cell dysfunction, ie, lipotoxicity (3). Chronic physiological increase in the plasma FFA concentration, eg, from 400 to 800 μM, decreases insulin-stimulated glucose disposal by ~25% in lean healthy normal glucose tolerant (NGT) individuals (4) and impairs beta cell function in genetically predisposed individuals, ie, the offspring of two diabetic individuals (5). Conversely, lowering the plasma FFA concentration with acipimox increases insulin sensitivity in T2DM individuals (6–8) and improves beta cell function in NGT (9) and T2DM (10) individuals.

Abbreviations:

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Chronic elevation in plasma glucose concentration also exerts a detrimental effect on both insulin sensitivity and insulin secretion, ie, glucotoxicity (11). We (12) and others (13) have demonstrated that a small persistent increase in plasma glucose concentration impairs both insulin-mediated nonoxidative glucose disposal and glucose-stimulated insulin secretion (14). Conversely, lowering the plasma glucose concentration in T2DM individuals improves both insulin sensitivity and beta cell function (15). The aim of the present study was to examine the effect of lowering both the plasma FFA concentration, with acipimox, and the plasma glucose concentration, with dapagliflozin, on insulin sensitivity and beta cell function in T2DM individuals.

RESEARCH DESIGN AND METHODS

Subjects

14 T2DM males (age = 50 ± 2 years; BMI = 32.7 ± 1.6 kg/m^2; HbA1c = 8.5 ± 0.3%; FPG = 186 ± 9 mg/dl; eGFR = 89 ± 6 ml/min.1.73m^2; diabetes duration = 6.3 ± 1.9 years) treated with metformin (n = 9) or metformin plus sulfonylurea (n = 5) participated in the study. Inclusion criteria included HbA1c = 7.0–10.0%, BMI = 24–40, eGFR ≥ 60 ml/min.1.73m^2, age = 18–70 years. Other than diabetes, subjects were in general good health as determined by medical history, physical examination, screening lab tests, urinalysis and EKG. Table 1 summarizes the clinical characteristics of the study participants. Body weight was stable (±3 pounds) in all subjects for ≥ 3 months prior to study and no subject participated in any excessively heavy exercise program. No subjects were taking any medications known to affect glucose metabolism other than metformin and sulfonylurea. The study protocol was approved by the IRB of the UTHSCSA and all subjects gave their written voluntary consent prior to participation.

Research design

After screening, eligible subjects received: (i) 2-hour 75-g oral glucose tolerance test (OGTT) and (ii) 4-hour hyperinsulinemic euglycemic clamp with 3-^{3}H-glucose to quantitate whole body insulin-mediated glucose disposal and endogenous glucose production (EGP). After completing the baseline studies, subjects received dapagliflozin (10 mg/d), an inhibitor of renal sodium glucose cotransporter 2 (SGLT2), for 22 days (from day 1 to 22). From days 15 to 22 subjects also received acipimox (250 mg, every 6 hours), an inhibitor of lipolysis, while continuing to take dapagliflozin. On days 13 and 14, and on days 21 and 22, the OGTT and insulin clamp were repeated.

Oral Glucose Tolerance Test (OGTT)

A 75-g OGTT was performed after a 10–12 hour overnight fast. Baseline blood samples for determination of plasma glucose, FFA, insulin and C-peptide concentrations were drawn at –30,-15, and 0 minutes. At time zero, subjects ingested 75 g of glucose in 300 ml of orange-flavored water, and plasma glucose, FFA, C-peptide and insulin concentrations were measured every 15 minutes for 2 hours.

Euglycemic Insulin Clamp

Subjects remained fasting after 10 PM on the night prior to study. At 6 AM on the following morning a cath-

Table 1. Metabolic characteristics of the diabetic subjects

	Baseline	Dapagliflozin	Dapagliflozin plus Acipimox	ANOVA
Weight (kg)	100.4 ± 4.9	99.3 ± 4.9	99.4 ± 4.9	NS
FPG (mg/dl)	186 ± 9	151 ± 8	131 ± 5	<0.001
2-h PG (mg/dl)	342 ± 16	269 ± 16	239 ± 13	<0.0001
Fasting C-peptide (ng/ml)	4.1 ± 0.4	4.3 ± 0.5	4.9 ± 0.5	NS
2-h C-Peptide (ng/ml)	8.7 ± 1.0	9.7 ± 0.9	11.0 ± 1.2	0.08
FPI (uU/ml)	9.2 ± 1.9	11.1 ± 2.4	8.5 ± 1.9	NS
2 h Plasma insulin (uU/ml)	32.2 ± 6.9	33 ± 4.8	31 ± 7	NS
Fasting FFA (mmol/liter)	0.52 ± 0.04	0.51 ± 0.04	0.36 ± 0.05	<0.05
bHGP (mg/kg ● min)	2.02 ± 0.10	2.40 ± 0.10	2.53 ± 0.15	<0.05
TGD (mg/kg ● min)	4.48 ± 0.50	5.30 ± 0.50	5.51 ± 0.34	<0.05
SSPI (uU/ml)	109 ± 5	107 ± 7	103 ± 5	NS
TGD/SSPI × 100	4.31 ± 0.61	5.07 ± 0.58	5.43 ± 0.61	<0.05
ΔG_{0-120} (mg/dl.h)	251 ± 12	199 ± 16	200 ± 12	<0.001
ΔI_{0-120} (μU/ml.h)	33 ± 9	35 ± 6	39 ± 7	NS
ΔC-Pep$_{0-120}$ (ng/ml.h)	5.6 ± 1.1	6.78 ± 1.0	8.8 ± 1.3	0.02
ΔC-Pep$_{0-120}/\Delta G_{0-120}$	0.019 ± 0.005	0.04 ± 0.005	0.05 ± 0.007	0.002
ΔC-Pep$_{0-120}/\Delta G_{0-120} \div$IR	0.09 ± 0.01	0.18 ± 0.03	0.22 ± 0.03	0.001

FPG = fasting plasma glucose; FPI = fasting plasma insulin; bHGP = basal rate of hepatic glucose production; TGD = total body glucose disposal rate; SSPI = steady state plasma insulin concentration; IR = insulin resistance

eter was placed into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into a vein on the dorsum of the hand, and the hand was placed into a thermoregulated box heated to 70°C. At 6 AM, a prime (25 μCi)-continuous (0.25 μCi/min) infusion of 3-^{3}H-glucose (DuPont NEN Life Science Products, Boston, MA) was started and continued for 7 hours. After a 3-hour basal tracer equilibration period (9 AM), subjects received a prime-continuous (80 mU/m^{2}min) insulin infusion for 240 minutes. During the last 30 minutes of the basal equilibration period, plasma samples were taken at 5–10 minute intervals for determination of plasma glucose and insulin concentrations and tritiated glucose radioactivity. During the insulin infusion, plasma glucose concentration was measured every 5 minutes. After the start of insulin no glucose was infused until the plasma glucose concentration declined to 100 mg/dl, at which level it was maintained by the adjustment of a variable glucose infusion. The plasma glucose concentration was maintained at ~100 mg/dl with a coefficient of variation < 5%. Plasma samples were collected every 15–30 minutes from 0 to 180 minutes after the start of insulin and every 5–10 minutes from 180 to 240 minutes for the determination of plasma glucose and insulin concentrations and tritiated glucose specific activity. Urine was collected from 0–240 minutes and urinary volume and glucose concentration were measured. Urinary glucose loss was subtracted from the total rate of glucose disposal to determine insulin-mediated tissue glucose uptake.

Analytical Techniques:

Plasma glucose was measured by the glucose oxidase reaction (Glucose Oxidase Analyzer, Analox, Fullerton, CA). Plasma FFA was measured spectrophotometrically (Wako, Neuss, Germany). Plasma insulin, C-peptide, and glucagon concentrations were measured by radioimmunoassay (RIA) (Linco Research, St. Louis, MO). Plasma 3-^{3}H-glucose radioactivity was measured in Somogyi precipitates.

Calculations and Statistical Analysis

Under steady-state postabsorptive conditions, the basal rate of endogenous (primarily reflects hepatic) glucose appearance (Ra) equals the 3-^{3}H-glucose infusion rate divided by steady state plasma tritiated glucose specific activity. After drug administration and during the insulin clamp, nonsteady conditions for 3-^{3}H-glucose specific activity prevail and the rate of glucose appearance (R_a) was calculated with Steele's equation. The rate of residual endogenous (primarily reflects hepatic) glucose production (HGP) during the insulin clamp was calculated by subtracting the exogenous glucose infusion rate from the tracer-derived R_a. The insulin-stimulated rate of total body glucose disposal (TGD) during the last hour of the insulin clamp (180–240 minutes) was calculated by adding the rate of residual HGP to the exogenous glucose infusion rate. The rate of tissue glucose uptake was calculated by subtracting the rate of urinary glucose excretion during the insulin clamp from the TGD rate. Hepatic insulin resistance was calculated as the product of the basal rate of HPG and the fasting plasma insulin concentration (16).

Insulin secretory rate (ISR) during the OGTT was calculated by deconvolution of the plasma C-peptide concentration curve (17), and the ratio between the incremental area under the plasma insulin secretory rate and incremental area under the plasma glucose concentration was calculated as previously described (18). β-cell glucose sensitivity, rate sensitivity, and the potentiation factor were calculated with the Mari model (17, 19). This model expresses glucose-stimulated insulin secretion (in pmol min^{-1} m^{-2}) as the sum of two components: (*i*) the first component represents the dependence of insulin secretion on the absolute plasma glucose concentration at any time point during the OGTT and is characterized by a dose-response function relating the two variables, the dose response slope being β-cell glucose sensitivity. The dose response is modulated by a potentiation factor that encompasses several glucose-dependent and glucose-independent potentiating mechanisms (eg, prolonged exposure to hyperglycemia, nonglucose substrates, gastrointestinal (GI) hormones, neural modulation, and molecular/biochemical/enzymatic changes within the β-cell). The second component represents the dependence of insulin secretion on the rate of change of plasma glucose, ie, the first derivative of plasma glucose concentration against time, and this parameter represents rate sensitivity. Rate sensitivity accounts for the observation that rapid changes in glucose concentration enhance insulin secretion more than slower changes in glucose concentration.

The incremental area under the plasma glucose and C-peptide concentration curves during the OGTT were calculated according to the trapezoid rule. Because both dapagliflozin and acipimox affect whole body insulin sensitivity, insulin secretion was related to the prevailing level of insulin resistance by calculating the insulin secretion/insulin resistance (IR) index, $\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120} \div IR$ (17, 18), to derive an index of beta cell function.

Values are expressed as mean ± SEM. The means at baseline and during dapagliflozin and dapagliflozin plus acipimox treatment were compared with ANOVA. Rates of TGD and EGP after dapagliflozin were compared to those before the start of dapagliflozin with paired *t* test. Statistical significance was set at alpha < 0.05.

Results

Treatment with dapagliflozin produced glucosuria which amounted to 78 g per 24 hours on mean and the glucosuria was maintained for 3 weeks. The induction of glucosuria with dapagliflozin caused a 35 mg/dl decrease in the fasting plasma glucose concentration (from 186 ± 9 to 151 ± 8 mg/dl at week 2, $P < .01$). Two weeks of dapagliflozin treatment did not affect the fasting plasma FFA concentration (0.52 ± 0.04 vs 0.51 ± 0.03 mM). Addition of acipimox to dapagliflozin caused a significant further decrease in the fasting plasma glucose concentration by 20 mg/dl ($P = .01$) and decreased the fasting plasma FFA concentration by 0.15 mM ($P < .05$ for both, Table 1).

Figure 1 depicts the plasma glucose concentration during the OGTT at baseline, at week 2 (treatment with dapagliflzoin) and at week 3 (treatment with dapagliflozin plus acipimox). Dapagliflozin significantly reduced both the 2-hour plasma glucose concentration and the incremental area under the plasma glucose concentration curve (ΔG_{0-120}) during the OGTT. Acipimox caused a significant reduction in both the FPG concentration (from 151 ± 8 to 131 ± 5, $P < .01$) and the 2-hour plasma glucose concentration (269 ± 16 to 239 ± 13 mg/dl, $P = .03$) (Table 2).

The fasting plasma insulin concentration was 10 ± 2 uU/ml and it slightly decreased with dapagliflozin treatment (8 ± 1 uU/ml, $P = .07$). After dapagliflozin plus acipimox treatment, there was a small insignificant decrease in the plasma insulin concentration (7 ± 1 mU/ml). The fasting plasma glucagon concentration was 125 ± 8 pg/ml at baseline and it significantly increased to 156 ± 11 pg/ml during dapagliflozin treatment ($P = .01$). There was an additional small nonsignificant increase in plasma glucagon concentration (169 ± 13, $P = .002$ vs baseline and p=NS vs treatment with dapagliflozin) during treatment with dapagliflozin plus acipimox. Thus, the ratio of plasma glucagon to insulin concentrations was 14 ± 4 at baseline and it rose to 23 ± 5 and 26 ± 4 during dapagliflozin and dapagliflozin plus acipimox, respectively.

Insulin Sensitivity. Consistent with previous studies from our lab (15), dapagliflozin treatment caused a significant increase in the basal rate of endogenous (hepatic) glucose production (bHGP) (from 2.02 ± 0.10 mg/kgmin to 2.40 ± 0.11 at week 2, $P < .01$). Following the addition of acipimox there was no significant change in bHGP at week 3 (2.53 ± 0.15, p=NS vs week 2). However, the basal rate of HGP remained significantly greater compared to baseline. Despite the increase in the basal rate of HGP with dapagliflozin, there was no significant change in the hepatic insulin resistance index (Table 1). The rate of total body insulin-stimulated glucose disposal increased significantly after dapagliflozin treatment by 17% ($P < .05$). Surprisingly, the addition of acipimox to dapagliflozin did not cause any further increase in the rate of insulin-stimulated total body glucose disposal (Table 1).

Insulin Secretion. Despite the decrease in FPG concentration caused by dapagliflozin, the basal insulin secretory rate increased slightly. Thus, the ratio between the basal insulin secretory rate and the fasting plasma glucose concentration significantly increased following dapagliflozin treatment (18 ± 2 to 25 ± 3, $P < .05$). The ratio between basal ISR and the fasting plasma glucose concentration was not further affected by the addition of acipimox to dapagliflozin (Table 2).

Dapagliflozin caused a significant decrease in the incremental area under the plasma glucose concentration curve during the OGTT (ΔG_{0-120}) and a significant increase in the total insulin output during the OGTT. Thus, the ratio between total insulin output and ΔG_{0-120} increased markedly following 2 weeks of dapagliflozin treatment ($P < .01$) (Table 2). Dapagliflozin also caused a significant increase in beta cell glucose sensitivity (Figure

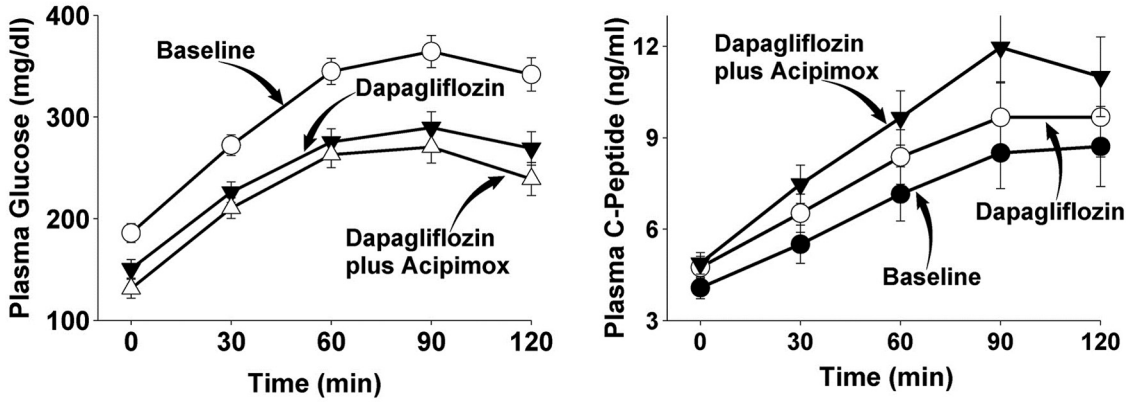

Figure 1. Plasma glucose (left) and C-peptide (right) concentrations during the OGTT performed at baseline, following dapagliflozin treatment for two weeks, and following combined dapagliflozin/acipimox treatment for one week.

Table 2. Effect of dapagliflozin and dapagliflozin plus acipimox on insulin secretion parameters

	Baseline	Dapagliflozin	Dapagliflozin +Acipmox	ANOVA
FPG (mg/dl)	186 ± 9	151 ± 8*	131 ± 5*$	<0.0001
Basal ISR	173 ± 19	202 ± 20	206 ± 21	NS
b-ISR/FPG	18 ± 2	25 ± 3+	28 ± 3#	<0.05
Rate sensitivity (pmol/m^2) ● (mmol/liter)	209 ± 70	327 ± 97	339 ± 132	NS
Glucose sensitivity (pmol●min/m^2) ● (mmol/liter)	23 ± 5	35 ± 5#	48 ± 8*$	<0.01
ISR at 135 mg/dl	134 ± 17	194 ± 23+	223 ± 21*$	<0.05
ISR at 180 mg/dl	191 ± 27	279 ± 31#	339 ± 34*$	<0.01
Total IS 0–120 (pmol)	42 ± 5	49 ± 5	58 ± 6#	<0.05
Total IS/mean PG	2.6 ± 0.4	3.7 ± 0.4#	4.6 ± 0.5*$	<0.01

FPG = fasting plasma glucose; IS = insulin secretion

+$P < 0.05$ *vs.* baseline

#$P < 0.01$ *vs.* baseline

*$P < 0.0001$ *vs.* baseline

\$$P < 0.05$ *vs.* dapagliflozin

3). Addition of acipimox to dapagliflozin had no significant effect on ΔG_{0-120}, but it caused a significant increase in total ISR during the OGTT. Thus, acipimox treatment produced a significant increase in the ratio between total insulin output and ΔG_{0-120} during the OGTT. Acipimox also caused a significant increase in beta cell glucose sensitivity ($P < .05$) (Figure 3). Beta cell glucose sensitivity strongly and inversely correlated with the fasting and mean plasma glucose concentrations during the OGTT following both dapagliflozin ($r = 0.60$, $P < .0001$) and combined acipimox/dapagliflozin ($r = 0.68$, $P < .0001$) treatnents (Figure 3b).

Because both dapagliflozin and acipimox have been shown to alter insulin sensitivity, it is necessary to relate insulin secretion to the prevailing level of insulin resistance. The insulin secretion/insulin resistance index ($\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120} \div IR$) increased 2-fold following dapagliflozin treatment (0.09 ± 0.01 to 0.18 ± 0.03, $P < .001$) and addition of acipimox to dapagliflozin caused a further 22% increase in $\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120} \div IR$ (0.18 ± 0.03 to 0.22 ± 0.03, $P < .05$)

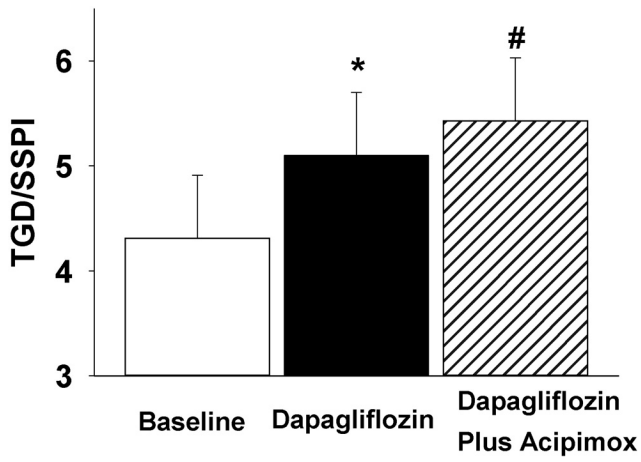

Figure 2. Insulin-mediated whole body tissue glucose during the euglycemic insulin clamp performed at baseline, following dapagliflozin treatment for two weeks, and following combined dapagliflozin/acipimox treatment for one week.

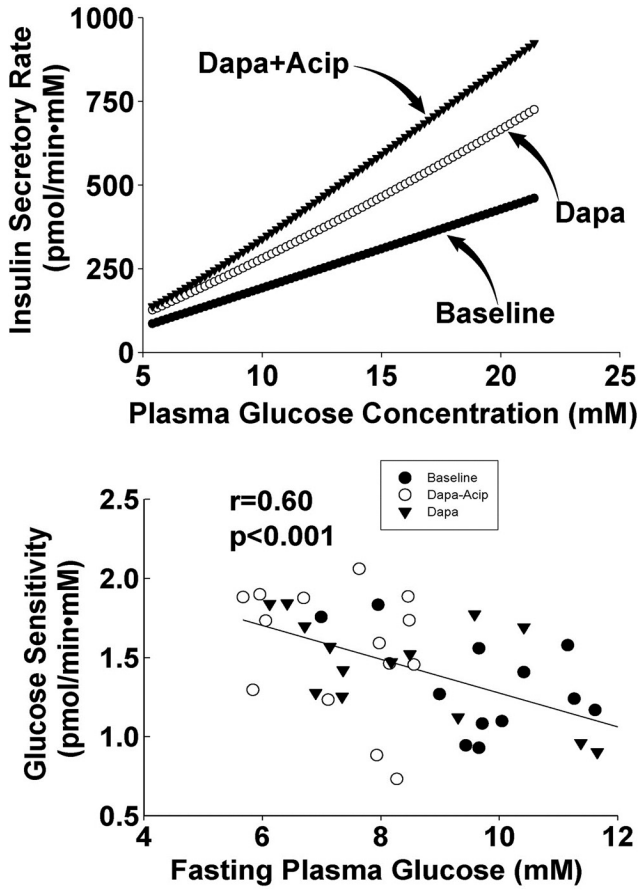

Figure 3. Beta cell glucose sensitivity (top panel) during the OGTT performed at baseline, following dapagliflozin treatment for two weeks, and following combined dapagliflozin/acipimox treatment for one week. Correlation between beta cell glucose sensitivity and fasting plasma glucose concentration on the day of the OGTT at baseline and following dapagliflozin and combined dapagliflozin/acipimox treatment (bottom panel).

Discussion

The results of the present study demonstrate that lowering the plasma glucose concentration with dapagliflozin improves both core defects, ie, insulin resistance and beta cell dysfunction, in T2DM. A decrease of 35 mg/dl in the fasting plasma glucose concentration was associated with a 17% increase in total body insulin-stimulated glucose disposal. Because the primary effect of dapagliflozin is to inhibit SGLT2 in the kidney and produce glucosuria (20), the increase in insulin-stimulated glucose disposal can be attributed to the decrease in plasma glucose concentration. Consistent with this, dapagliflozin caused no significant change in the fasting plasma free fatty acid concentration. Previous studies have documented a glucotoxic effect of chronic hyperglycemia to impair insulin action in muscle in experimental animals and in man (15, 21–27). The results of the present study are consistent with these prior observations and extend them by demonstrating that chronic elevation of the plasma glucose concentration has a differential effect on insulin resistance in skeletal muscle vs liver. Thus, despite a 17% improvement in insulin-stimulated total body glucose disposal following dapagliflozin treatment, reduction in the plasma glucose concentration had no significant effect on the hepatic insulin resistance index in diabetic individuals. The improvement in peripheral tissue insulin sensitivity following dapagliflozin treatment underscores the importance of improving glycemic control in T2DM patients.

Treatment with dapagliflozin alone and with dapagliflozin plus acipimox resulted in a significant decrease in the plasma glucose concentration and a paradoxical increase in the basal rate of HGP. Because of the strong correlation between bHGP and the fasting plasma glucose concentration, this finding may appear paradoxical. One possible explanation is that dapagliflozin treatment increased the basal rate of glucose clearance. Indeed, glucose clearance (measured as bHGP/FPG) increased significantly during the treatment period. However, because dapagliflozin increased urinary glucose loss, tissue glucose clearance (after subtraction of urinary glucose loss) was not significantly altered by dapagliflozin treatment, either with or without acipimox.

The present results also provide evidence for a glucotoxic effect of chronic hyperglycemia on beta cell function. Reduction of the plasma glucose concentration from 186 to151 mg/dl with dapagliflozin was associated with a doubling of the insulin secretion/insulin resistance index ($\Delta C\text{-}Pep_{0-120}/\Delta G_{0-120} \div IR$). Because SGLT2 transporters are not present in beta cells, because the primary effect of dapagliflzoin is on the kidney to produce glucosuria and lower the plasma glucose concentration, and because the plasma FFA concentration did not change following dapagliflozin treatment, it is plausible to conclude that the beneficial effect of lowering the plasma glucose concentration resulted from the amelioration of glucotoxicity. Dapagliflozin improved total body insulin sensitivity and reduced the incremental area under the plasma glucose concentration curve during the OGTT. Both of these actions of dapagliflozin would be anticipated to decrease the absolute amount of insulin secreted during the OGTT. In contrast, total insulin output during the OGTT significantly increased following dapagliflozin treatment, emphasizing the powerful effect of reducing the plasma glucose concentration to augment beta cell function. The increase in total insulin output during the OGTT was strongly related to the increase in beta cell glucose sensitivity ($r = 0.80$, $P < .0001$) which, in turn, strongly correlated with the decline in both fasting plasma glucose concentration ($r = 0.60$, $P < .0001$) and mean plasma glucose concentration during the OGTT ($r = 0.68$, $P < .0001$). These findings provide strong support for an important pathophysiologic role for glucotoxicity in the development of beta cell failure in T2DM.

Acipimox is a powerful inhibitor of lipolysis, leading to a reduction in plasma FFA concentration (6–8) and has been shown to improve tissue sensitivity to insulin and beta cell function in T2DM individuals (6–8). These findings have been interpreted as evidence for a role of elevated plasma FFA in the development of insulin resistance, ie, lipotoxicity. However, in all previous studies, as well as in the present study, acipimox also lowered the plasma glucose concentration. Thus, removal of the detrimental effect of chronic elevation in plasma glucose concentration on insulin sensitivity and beta cell function could have contributed to the effect of acipimox to ameliorate insulin resistance and improve beta cell dysfunction. In the present study, acipimox lowered the plasma FFA concentration and, when added to dapagliflozin, it caused a further decrease in the plasma glucose concentration. Importantly, it caused an additional improvement in beta cell function beyond that caused by dapagliflozin alone. It is noteworthy that, while acipimox caused a small increase in both the fasting and 2-hour plasma C-peptide concentrations during the OGTT and a large increase in $\Delta Cpep_{(0-120)}$, it caused a small decrease in both the fasting and the 2-hour plasma insulin concentrations and a much smaller increase in $\Delta I_{(0-120)}$. This could be explained by the increase in plasma insulin clearance, although the increase did not reach statistical significance. This observation underscores the importance of measuring C-peptide in the assessment of beta cell function.

Surprisingly, acipimox failed to cause a further improvement in insulin sensitivity when added to dapagli-

flozin in T2DM individuals. Although the plasma FFA concentration did not change following dapagliflozin therapy, previous studies have reported an increase in lipid oxidation following SGLT2 inhibition without any change in plasma FFA concentration (16). Thus, it is possible that the intramyocelluar concentration of toxic lipid metabolites declined with dapagliflozin treatment. This could explain why acipimox, when added to dapagliflozin, failed to further increase insulin-stimulated glucose disposal (29). It also is possible that the primary mechanism by which acipimox improves insulin sensitivity is via a reduction in plasma glucose concentration and that the decrease in plasma FFA concentration makes only a minor contribution to the improvement in insulin sensitivity. Previous studies with the euglycemic hyperinsulinemic clamp (32) have documented that axipimox treatment improves whole body insulin sensitivity, independent of the change in plasma FFA concentration. Thus, it is possible that the reduction in plasma glucose concentration with acipimox, not the decrease in plasma FFA concentration, is the major mechanism by which acipimox improves whole body insulin sensitivity. If this scenario is correct, then improvement of glucotoxicity by dapagliflozin prior to acipimox treatment could explain the lack of significant increase in insulin sensitivity by acipimox.

Consistent with previous results (15, 16), dapagliflozin treatment was associated with a significant increase in the basal rate of EGP which correlated with the increase in plasma glucagon concentration. The increase in plasma glucagon can be explained, at least in part, by inhibition of the SGLT2 transporter in the alpha cell (30).

There are several limitations of the present study. First, the present study included only males. Therefore, extrapolation of the results to females should be done with caution. A second limitation is the lack of a placebo-treated group for the addition of acipimox. Although changes in diet, physical activity or body weight could have affected the results, body weight did not change significantly and, by history, the participants did not change their dietary intake or daily physical activity regimen. It also is possible that the improvement in beta cell function observed with acipimox treatment was due to longer exposure to dapagliflozin (2 weeks vs 3 weeks), and not due to acipimox. This is an unlikely scenario, because a recent study (16) which assessed the effect of SGLT2 inhibition on beta cell function reported a similar beneficial effect of SGLT2 inhibition with empagliflozin on beta cell function on day 1 vs week 4 after the start of the drug. Lastly, it would be of interest to examine whether the beneficial effects of dapagliflozin and acipimox persist with longer duration of therapy.

In summary, induction of glucosuria and resultant decline in plasma glucose concentration with dapagliflozin markedly enhanced beta cell function ($\Delta I/\Delta G \div IR$) and beta cell sensitivity to glucose (Mari model). Addition of acipimox to dapagliflozin further improved beta cell function (insulin secretion/insulin resistance index) and beta cell sensitivity to glucose, emphasizing the importance of correcting both the disturbances in glucose and FFA metabolism in the restoration of beta cell function in poorly controlled type 2 diabetic patients. The strong association between the decrease in plasma glucose concentration and increase in beta cell sensitivity to glucose emphasizes the important and deleterious effect of glucotoxicity in the etiology of beta cell failure in T2DM. Thus, hyperglycemia is, not only a manifestation of the diabetic state and responsible for the microvascular complications, but also a self-perpetuating cause of the diabetic condition.

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Address all correspondence and requests for reprints to: Ralph A DeFronzo, MD, Division of Diabetes, UTHSCSA, 7703 Floyd Curve Dr. San Antonio, TX, 78 229, Phone (210) 567 6691, Fax (210) 567 6554, albarado@uthscsa.edu.

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*contributed equally to completion of the study

CONFLICT OF INTEREST: AM, MAG, AM, CS, JX, GD, DT have no conflicts of interest. RAD - Advisory Board: Astra Zeneca, Novo Nordisk, Janssen, Lexicon, Boehringer-Ingelheim; Research Support: Bristol Myers Squibb, Boehringer-Ingelheim, Takeda, Astra Zeneca; Speaker's Bureau: Novo-Nordisk, Astra Zeneca.

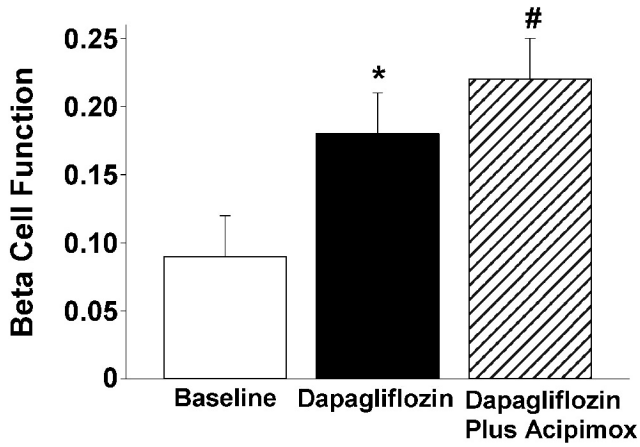

Figure 4. Insulin secretion/insulin resistance (disposition) index at baseline and following dapagliflozin and combined dapagliflozin/acipimox treatment (bottom panel).

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