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Shift to Fatty Substrate Utilization in Response to Sodium–Glucose Cotransporter 2 Inhibition in Subjects Without Diabetes and Patients With Type 2 Diabetes

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Pharmacologically induced glycosuria elicits adaptive responses in glucose homeostasis and hormone release. In type 2 diabetes (T2D), along with decrements in plasma glucose and insulin levels and increments in glucagon release, sodium–glucose cotransporter 2 (SGLT2) inhibitors induce stimulation of endogenous glucose production (EGP) and a suppression of tissue glucose disposal (TGD). We measured fasting and postmeal glucose fluxes in 25 subjects without diabetes using a double glucose tracer technique; in these subjects and in 66 previously reported patients with T2D, we also estimated lipolysis (from [$^2H_5$]glycerol turnover rate and circulating free fatty acids, glycerol, and triglycerides), lipid oxidation (LOx; by indirect calorimetry), and ketogenesis (from circulating β-hydroxybutyrate concentrations). In both groups, empagliflozin administration raised EGP, lowered TGD, and stimulated lipolysis, LOx, and ketogenesis. The pattern of glycosuria-induced changes was similar in subjects without diabetes and in those with T2D but quantitatively smaller in the former. With chronic (4 weeks) versus acute (first dose) drug administration, glucose flux responses were attenuated, whereas lipid responses were enhanced; in patients with T2D, fasting β-hydroxybutyrate levels rose from 246 ± 288 to 561 ± 596 μmol/L ($P < 0.01$). We conclude that by shunting substantial amounts of carbohydrate into urine, SGLT2-mediated glycosuria results in a progressive shift in fuel utilization toward fatty substrates. The associated hormonal milieu (lower insulin-to-glucagon ratio) favors glucose release and ketogenesis.

When large quantities of glucose are pharmacologically forced into urinary excretion, whole-body metabolism undergoes adaptive changes involving glucose fluxes, hormonal responses, fuel selection, and energy expenditure (1,2). In previous work (3), we used empagliflozin to investigate the physiological response to forced glycosuria in patients with type 2 diabetes (T2D). By combining a mixed meal with the double-tracer technique, we found that after acute or chronic empagliflozin administration endogenous glucose production (EGP) rose, tissue glucose disposal (TGD) decreased, and lipid utilization increased. The aims of the present work were to measure the full spectrum of changes in lipolysis, lipid levels, and substrate availability consequent upon empagliflozin-induced glycosuria in patients with T2D and to test whether and to what extent these changes occur in subjects without diabetes.

RESEARCH DESIGN AND METHODS

Population

Sixty-six patients with T2D were recruited into the study; their inclusion criteria are detailed in Ferrannini et al. (3). Twenty-five subjects without diabetes (12 with normal glucose tolerance [NGT] and 13 with impaired glucose tolerance [IGT]) served as control subjects (Supplementary Table 1). The glucose and hormone data for the patients with T2D have been reported (3) and are repeated here for comparison purposes. The study (clinicaltrials.gov identifier NCT01248364; EudraCT number 2010-018708-99)

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was carried out at three sites (Pisa, Italy; Neuss, Germany; and Graz, Austria); the protocol was approved by the institutional review board at each site. All participants provided informed written consent.

Design

Participants with T2D and IGT underwent three open-label studies: baseline, acute (single dose of 25 mg empagliflozin), and chronic (25 mg/day for 28 days); subjects with NGT did not participate in the chronic study. Each study consisted of a 5-h meal tolerance test after a 3-h basal period combined with a double-tracer technique (3). A primed (1.5 $\mu mol \cdot kg^{-1}$) constant (0.1 $\mu mol \cdot min^{-1} \cdot kg^{-1}$) infusion of [$^2H_5$]glycerol (Cambridge Isotope Laboratories, Boston, MA) was administered throughout the test, starting at time −180 min. The meal consisted of one egg, 50 g of parmesan cheese, 50 g of white bread, and 75 g of glucose in water. In both studies, empagliflozin was ingested 30 min before starting tracer infusion (at time –210 min). Blood was drawn at timed intervals for the measurement of tracers, hormones, and substrates. Indirect calorimetry was performed for periods of 30 min at fixed intervals (3). Urine was collected separately during the basal period and during the meal.

Measurements

All measurements were performed at the Metabolism Lab at the University of Pisa; samples from all three studies of each subject were assayed together to reduce intrasubject variability. Tracer enrichments were determined by gas chromatography/mass spectrometry as previously described (3,4).

Plasma glucose, free fatty acids (FFAs), lactate, glycerol, and β-hydroxybutyrate concentrations were measured on a Synchron system CX4 (Beckman Instruments, Fullerton, CA). Plasma insulin and C-peptide were assayed on a COBAS e411 (Roche, Indianapolis, IN), glucagon was measured by radioimmunoassay, and total COOH-terminal amidated glucagon-like peptide 1 (GLP-1) was measured by ELISA (Millipore Corporation, Billerica, MA).

Calculations

Glucose fluxes were expressed per kilogram of fat-free mass (5). Glucose and glycerol clearance rate and fluxes were computed using the circulatory model (6). As detailed in Ferrannini et al. (3), the model yields fasting and postmeal EGP and oral glucose appearance (RaO). TGD was obtained as the difference between model-derived total rate of glucose disappearance and urinary glucose excretion. The prehepatic insulin-to-glucagon molar concentration ratio was calculated as previously described (3). Glucose oxidation (GOx), lipid oxidation (LOx), protein oxidation, and nonprotein respiratory quotient were measured by indirect calorimetry (7). Nonoxidative glucose disposal was calculated as the difference between TGD and GOx.

Area under the curve (AUC) was calculated (by the trapezium rule) for the 3-h basal fasting period and the 5-h postmeal period; mean values were obtained by dividing the AUC by the corresponding time interval.

β-Cell Function Modeling

The model used to reconstruct insulin secretion and its control by glucose has been previously described (8). The main model-derived parameters are β-cell glucose sensitivity and rate sensitivity. Total insulin secretion is the sum of these two components.

Statistical Analysis

Data are given as mean ± SD (or median [interquartile range] for nonnormally distributed variables). Acute and chronic treatment responses were compared with the respective baseline responses by paired Student *t* test or Wilcoxon signed rank test depending on the underlying data distribution. For the primary analysis, we compared patients with T2D with all 25 subjects without diabetes (i.e., NGT+IGT) because of the overall similarity of results in subjects with NGT and IGT; separate data for subjects with NGT and IGT are presented as appropriate. Group differences were tested by unpaired Student *t* test or Mann-Whitney *U* test. A two-tailed *P* value of ≤ 0.05 was considered statistically significant.

RESULTS

Subjects without diabetes were younger and heavier than patients with T2D. At baseline, fasting levels of insulin, glucagon, GLP-1, and glucose-dependent insulinotropic peptide were similar in the two groups (Supplementary Table 1). During the meal, glucose was lower but insulin was higher in subjects without diabetes than in those with T2D; consequently, both β-cell glucose sensitivity and rate sensitivity were higher in subjects without diabetes. Also, as meal-stimulated glucagon release was similar, the postmeal insulin-to-glucagon ratio was 50% higher in subjects without diabetes (Supplementary Table 2). Fasting EGP was lower in subjects without diabetes than in those with T2D; on the meal, RaO was similar but TGD was larger in subjects without diabetes, likely due to their higher insulin levels (Table 1).

On empagliflozin, similar changes were seen in subjects with T2D and in those without diabetes: fasting and postmeal glucose and insulin levels decreased and postmeal glucagon and GLP-1 increased, the insulin-to-glucagon ratio and insulin secretion decreased, and β-cell glucose sensitivity improved (Supplementary Tables 1 and 2). In addition, EGP rose and TGD declined (Table 1). In both groups, during chronic versus acute treatment, the increments in glucagon and GLP-1 were attenuated, whereas the decrease in TGD was accentuated; the changes in insulin secretion and β-cell glucose sensitivity were maintained. Differences between the two groups were the amount of glycosuria (approximately one-third lower in subjects without diabetes than in those with T2D) (Supplementary Table 3) and fasting EGP, which was lower in subjects without diabetes than in those with T2D (Supplementary Fig. 1).

Table 1—Glucose fluxes

	T2D			ND		
	Baseline	Acute	Chronic	Baseline	Acute	Chronic*
Fasting EGP (μmol · kg_{FFM}^{-1} · min^{-1})	13.8 (5.2)	17.6 (4.8)¶	17.5 (4.1)¶	10.7 (3.4)§	14.3 (3.4)¶§	13.8 (5.2)¶§
RaO_{AUC} (g · h)	61 (14)	62 (15)	63 (12)	58 (15)	57 (11)	59 (19)
EGP_{AUC} (g · h)	34 (11)	40 (14)¶	37 (11)¶	42 (15)§	43 (10)	43 (12)
TGD_{AUC} (g · h)	93 (18)	75 (16)¶	70 (21)¶	103 (21)§	84 (17)¶§	72 (31)¶

Data are median (interquartile range). The AUC is during the 5-h postmeal period. FFM, fat-free mass. *IGT only (n = 13). ¶$P \leq 0.05$ vs. respective baseline value by paired Student t test or Wilcoxon signed rank test. §$P \leq 0.05$ vs. T2D by Mann-Whitney U test.

In T2D at baseline, fasting β-hydroxybutyrate levels were halved during the meal; on empagliflozin, however, they rose significantly (peaking at 60 min), especially with chronic treatment (Fig. 1). Plasma actate levels declined during the 3-h fasting period, peaked 2 h into the meal, and then returned to premeal levels at 5 h. On empagliflozin, fasting and postmeal lactate declined with chronic treatment (Table 2). Fasting plasma glycerol and FFA concentrations were strongly suppressed during the meal; on empagliflozin, mean postmeal glycerol and fasting and postmeal FFAs were higher than at baseline, especially on chronic treatment (Supplementary Fig. 2). Fasting glycerol R_a was suppressed by 35% during the meal; with empagliflozin, glycerol R_a was slightly, but not significantly, increased (Fig. 2). LOx was increased and GOx and nonoxidative disposal were reduced (Supplementary Table 3) after chronic empagliflozin administration.

In subjects without diabetes, the pattern of changes was similar to that in patients with T2D except that at baseline, fasting and postmeal β-hydroxybutyrate and lactate levels were lower but plasma glycerol and FFAs and glycerol R_a were not significantly different. With empagliflozin, β-hydroxybutyrate (fasting and postmeal)

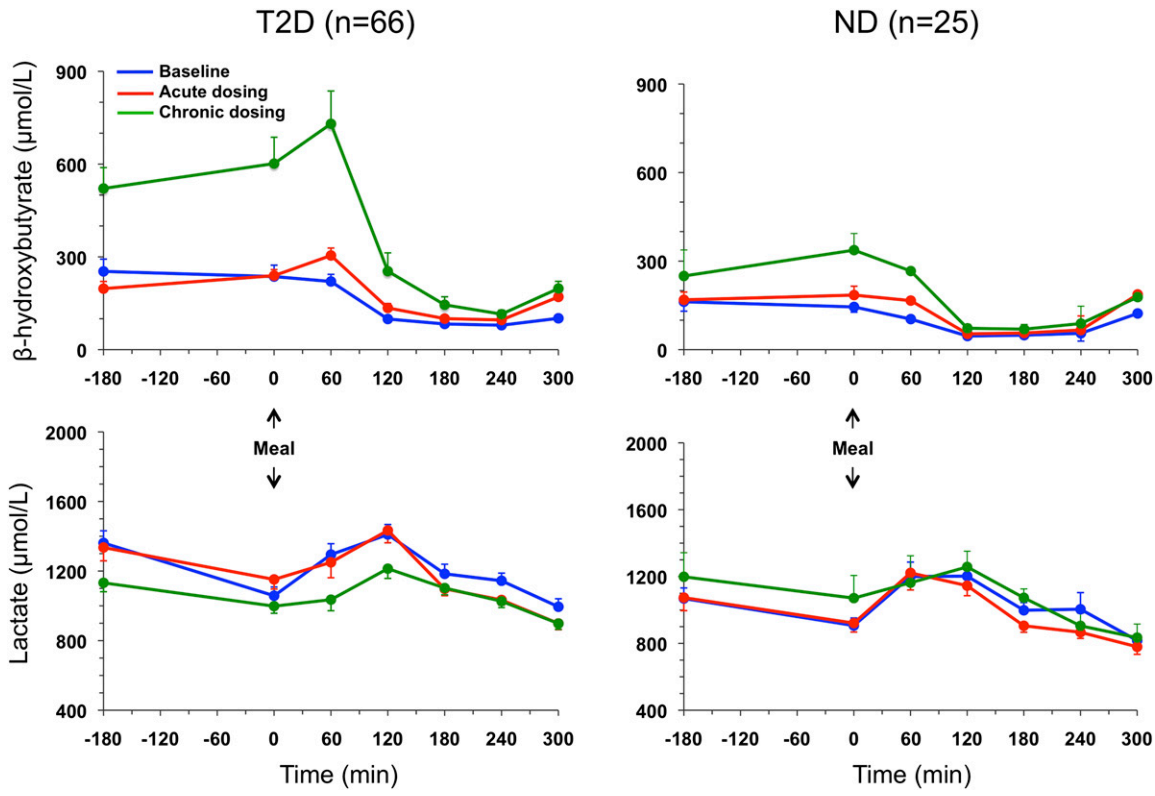

Figure 1—Plasma β-hydroxybutyrate and lactate concentrations during the 3 h of fasting and the 5 h after meal ingestion at baseline and after acute and chronic empagliflozin administration in patients with T2D (n = 66) and subjects without diabetes (ND, n = 25). Data after chronic dosing in subjects without diabetes are from subjects with IGT only (n = 13). Empagliflozin was administered at time –210 min. Plots are mean ± SEM.

Table 2—Circulating substrates

	T2D			ND		
	Baseline	Acute	Chronic	Baseline	Acute	Chronic*
Fasting β-hydroxybutyrate (μmol/L)#	246 ± 288	220 ± 150	561 ± 596¶	145 ± 138§	173 ± 109	267 ± 207§
Meal β-hydroxybutyrate$_{mean}$ (μmol/L)	133 ± 96	172 ± 94¶	330 ± 382¶	77 ± 43§	109 ± 65¶§	151 ± 93§
Fasting lactate (mmol/L)#	1.42 ± 0.42	1.30 ± 0.39¶	1.20 ± 0.40¶	1.07 ± 0.32§	1.06 ± 0.43§	1.11 ± 0.58
Meal lactate$_{mean}$ (mmol · L^{-1} · 5h)	1.55 ± 0.49	1.50 ± 0.54	1.32 ± 0.46¶	1.06 ± 0.23§	0.96 ± 0.31§	1.07 ± 0.33§
Fasting glycerol (μmol/L)#	112 ± 31	103 ± 36	115 ± 39	116 ± 76	118 ± 89	108 ± 29
Meal glycerol$_{mean}$ (μmol/L)	80 ± 20	83 ± 20¶	86 ± 23¶	77 ± 24	85 ± 26¶	93 ± 36¶
Fasting FFA (μEq/L)#	605 ± 172	520 ± 145¶	652 ± 182¶	535 ± 181	496 ± 217	611 ± 143
Meal FFA$_{mean}$ (μEq/L)	225 ± 76	287 ± 97¶	315 ± 110¶	194 ± 70	234 ± 80¶§	271 ± 89
Fasting glycerol R_a (μmol · kg^{-1} · min^{-1})	2.9 ± 1.2	3.0 ± 1.1	3.1 ± 1.2	2.7 ± 0.6	3.2 ± 1.4¶	3.5 ± 0.8¶
Meal glycerol Ra$_{mean}$ (μmol · kg^{-1} · min^{-1})	1.9 ± 0.6	1.9 ± 0.6	2.0 ± 0.6	2.1 ± 0.6	2.2 ± 0.7§	2.5 ± 1.0§

Data are median (interquartile range). Mean is the average value during the 5-h postmeal period. ND, subjects without diabetes. *IGT only (n = 13). #Before drug administration. ¶$P \leq 0.05$ vs. respective baseline by Wilcoxon signed rank test. §$P \leq 0.05$ vs. T2D by Mann-Whitney U test.

tended to increase, lactate did not change, and postmeal glycerol levels and fasting glycerol R_a values increased. The changes in glucose and LOx were qualitatively similar to those in patients with T2D but did not reach statistical significance.

Serum Lipids

During the meal, triglycerides rose in both groups. With empagliflozin, fasting lipids generally changed minimally. A small but statistically significant increase in triglycerides was observed the acute study both in subjects with T2D

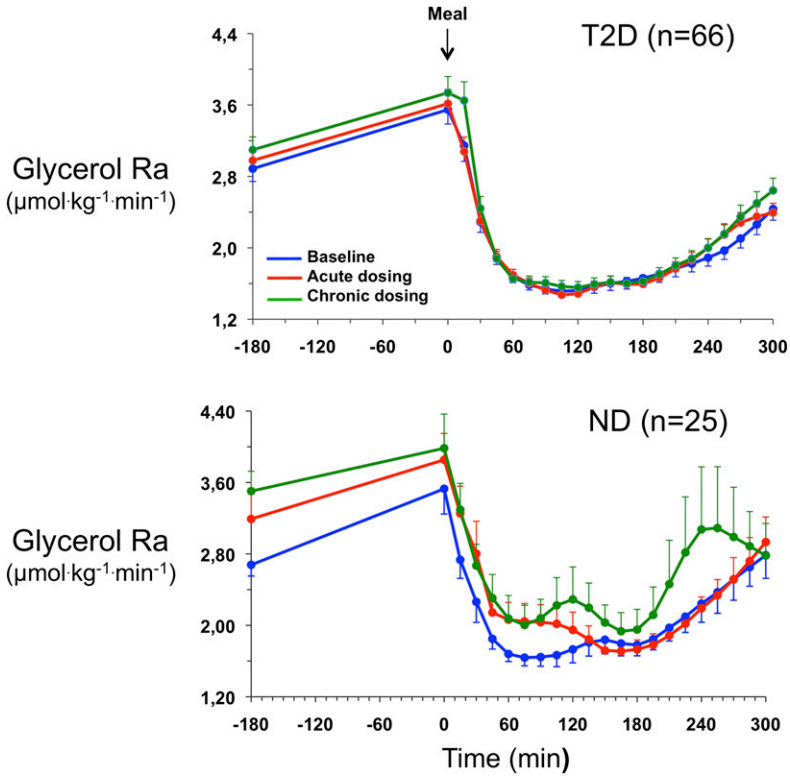

Figure 2—Rates of systemic glycerol appearance (R_a) during the 3 h of fasting and the 5 h after meal ingestion at baseline after acute and chronic empagliflozin administration in patients with T2D (n = 66) and subjects without diabetes (ND, n = 25). Data after chronic dosing in subjects without diabetes are from subjects with IGT only (n = 13). Empagliflozin was administered at time –210 min. Plots are mean ± SEM.

and those without diabetes, which subsided with chronic treatment (Supplementary Table 4).

DISCUSSION

Sodium–glucose cotransporter 2 (SGLT2)–induced glycosuria engenders a chain of metabolic adaptations to the ensuing decrease in plasma glucose concentrations: increased EGP, reduced TGD and GOx, accelerated lipolysis, increased fat oxidation, and enhanced ketogenesis. Higher FFA and glycerol levels track the stimulated lipolysis, lower lactate levels signal the reduced carbohydrate oxidation (as well as augmented hepatic uptake for use in gluconeogenesis), and higher β-hydroxybutyrate levels reflect the enhanced liver fat oxidation. Hormonal mediation is provided by a drop in insulin secretion and an increase in glucagon release. Thus, in response to persistent dumping of glucose into the urine, whole-body metabolism shifts to enhanced usage of fat for energy production. Overall, the resulting pattern is akin to that of fasting except that fasting develops more slowly. In the longer run, the loss of glucose calories concurs with the increased lipid mobilization to cause loss of fat mass and weight (2).

The pattern of metabolic changes was not limited to patients with T2D but also was observed in individuals without diabetes, with some attenuations. Specifically, in subjects with IGT, ketogenesis, as reflected in fasting and postmeal β-hydroxybutyrate levels, increased less than in T2D patients after chronic treatment. In the baseline study, plasma β-hydroxybutyrate was lower in subjects without diabetes than in those with T2D (Fig. 1) and rose less in IGT subjects than in T2D patients with chronic treatment. In the whole cohort, fasting β-hydroxybutyrate correlated positively with fasting plasma glucose ($r = 0.24$, $P = 0.02$) and FFA levels ($r = 0.33$, $P < 0.002$). In addition, across all studies, fat oxidation represented a greater percentage of total substrate oxidation in subjects with T2D than in those without diabetes, both in the fasting state (0.50% [0.30] vs. 0.45% [0.31], $P < 0.04$) and postmeal (0.43% [0.4] vs. 0.36% [0.33], $P < 0.04$). These differences indicate that even well-controlled patients with T2D rely more on fatty substrate utilization than do individuals without diabetes, whereby ketogenesis is chronically upregulated in a degree roughly proportional to the severity of hyperglycemia.

Performing both acute and chronic studies made it possible to delineate a time sequence of the metabolic changes. Thus, the glycosuria and the decrement in plasma glucose began to manifest very shortly (within hours) after the first empagliflozin dose, and the hormonal changes (insulin, glucagon, and GLP-1) and EGP followed suit. In contrast, the increase in lipolysis, the shift in substrate utilization, and the rise in β-hydroxybutyrate concentrations became more marked after chronic treatment. The empagliflozin-induced hormonal changes were qualitatively similar in subjects with T2D and in those without diabetes, although the decline in insulin was persistent, whereas the rise in glucagon and GLP-1 was attenuated over 4 weeks of treatment. The relative hyperglucagonemia can be accounted for by lower glycemia and insulin release; insulin normally restrains glucagon secretion by a paracrine mechanism (9). Moreover, recent in vitro and ex vivo work has suggested that inhibition of SGLT2 with dapagliflozin in pancreatic α-cells directly triggers glucagon secretion (10). While the greater GLP-1 response, albeit small, should have suppressed glucagon release, the drop in the prehepatic insulin-to-glucagon ratio prevailed in raising EGP; the rise in circulating FFA levels (which stimulate gluconeogenesis) (11,12) presumably provided a further positive stimulus to EGP.

With empagliflozin dosing, postmeal triglycerides rose acutely and tended to decline toward baseline with chronic dosing. The changes in fasting LDL and HDL cholesterol are similar to those recently reported in the BI 10773 (Empagliflozin) Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) trial (13), which showed a significant reduction in incident major cardiovascular disease outcomes.

The stimulation of ketogenesis observed during treatment with SGLT2 inhibitors has raised concern of so-called euglycemic ketoacidosis (14–17). The current data provide a pathophysiological framework for this occurrence. In our group with T2D, fasting β-hydroxybutyrate levels, which were higher than in subjects without diabetes, ranged from 1.0 to 1.5 mmol/L in only four individuals at baseline but in 11 out of 66 individuals after chronic treatment. After the meal, there was a transient further rise in on-treatment β-hydroxybutyrate levels, which ranged 1.2–4.9 mmol/L in 11 patients. Thus, mild, transient ketosis, without symptoms or signs of acidosis, was not infrequent even in this group of well-controlled patients with T2D. Upon comparing subjects with T2D and IGT in whom fasting β-hydroxybutyrate while on chronic empagliflozin treatment fell in the top quartile of the distribution (range 0.67–3.5 mmol/L) to the rest of the cohort (Supplementary Table 5), we found that at baseline, these subjects had significantly lower fasting and postmeal plasma insulin and β-hydroxybutyrate levels and worse β-cell insulin sensitivity.

We do not know whether increased calorie intake, which eventually balances out urinary calorie loss and causes weight loss to cease (18), would entirely reverse the metabolic shift observed here. However, classical triggering factors may advance ketosis into euglycemic ketoacidosis (19). The presence of sizeable endogenous insulin secretion generally protects the average patient with T2D against these precipitating factors, but an excessive, brisk reduction in exogenous insulin doses may enhance the risk of ketosis/ketoacidosis in patients with diabetes on treatment with SGLT2 inhibitors.

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Author Contributions. E.F. and E.M. designed experiments, analyzed data, and wrote the manuscript. S.B., S.F., and B.A. carried out all laboratory determinations. T.H. and T.R.P. performed in vivo experiments and contributed to discussions. R.B. and A.M. performed all modeling analyses. All authors edited the manuscript. E.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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