Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients

Ele Ferrannini, …, Uli C. Broedl, Hans-Juergen Woerle


**Background.** Sodium-glucose cotransporter 2 (SGLT2) inhibitors lower glycemia by enhancing urinary glucose excretion. The physiologic response to pharmacologically induced acute or chronic glycosuria has not been investigated in human diabetes.

**Methods.** We evaluated 66 patients with type 2 diabetes (62 ± 7 years, BMI = 31.6 ± 4.6 kg/m$^2$, HbA$_1c$ = 55 ± 8 mmol/mol, mean ± SD) at baseline, after a single dose, and following 4-week treatment with empagliflozin (25 mg). At each time point, patients received a mixed meal coupled with dual-tracer glucose administration and indirect calorimetry.

**Results.** Both single-dose and chronic empagliflozin treatment caused glycosuria during fasting (median, 7.8 [interquartile range {IQR}, 4.4] g/3 hours and 9.2 [IQR, 5.2] g/3 hours) and after meal ingestion (median, 29.0 [IQR, 12.5] g/5 hours and 28.2 [IQR, 15.4] g/5 hours). After 3 hours of fasting, endogenous glucose production (EGP) was increased 25%, while glycemia was 0.9 ± 0.7 mmol/l lower ($P < 0.0001$ vs. baseline). After meal ingestion, […]

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Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients

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Introduction

Under normal circumstances, glycosuria is minimal, even after a large meal, because plasma glucose concentrations rarely exceed the renal glucose threshold. In persons with diabetes, glycosuria provides some protection against severe hyperglycemia. Sodium-glucose cotransporter 2 (SGLT2), a low-affinity, high-capacity member of an increasing family of cotransporters (1), is highly expressed in the proximal renal tubule, in which it is reputed to be responsible for the bulk of reabsorption of filtered glucose (2). The idea of blocking SGLT2 activity to induce therapeutic glycosuria stems from the first demonstration of competitive inhibition by phlorizin of glucose uptake into brush border vesicles from normal human kidney (3). Based on these premises, a new class of orally active antihyperglycemic agents has recently undergone pharmacological development, with some of them already available for clinical use (11–16). Most of these compounds are highly selective for SGLT2, although dual SGLT2/SGLT1 inhibitors also are being tested for the treatment of type 2 diabetes (17, 18).

Conflict of interest: This study was sponsored by Boehringer Ingelheim. Elle Ferrannini has served as an ad hoc consultant and occasional speaker for Boehringer Ingelheim, Merck & Co., Sanofi, Eli Lilly and Co., Johnson & Johnson, Astellas, Daiichi Sankyo, Bristol-Myers Squibb/AstraZeneca, and Novartis. Andrea Mari has received research grants from Eli Lilly and Co. and Novo Nordisk. Tim Heise is an employee of Profil. Uli C. Broedl and Hans-Juergen Woerle are employees of Boehringer Ingelheim.

Citation for this article: J Clin Invest. 2013;124(2):499–508. doi:10.1172/JCI72227.

Methods

We evaluated 66 patients with type 2 diabetes (62 ± 7 years, BMI = 31.6 ± 4.6 kg/m², HbA1c = 55 ± 8 mmol/mol, mean ± SD) at baseline, after a single dose, and following 4-week treatment with empagliflozin (25 mg). At each time point, patients received a mixed meal coupled with dual-tracer glucose administration and indirect calorimetry.

Results. Both single-dose and chronic empagliflozin treatment caused glycosuria during fasting (median, 7.8 [interquartile range [IQR], 4.4] g/3 hours and 9.2 [IQR, 5.2] g/3 hours) and after meal ingestion (median, 29.0 [IQR, 12.5] g/5 hours and 28.2 [IQR, 15.4] g/5 hours). After 3 hours of fasting, endogenous glucose production (EGP) was increased 25%, while glycosuria was 0.9 ± 0.7 mmol/lower (P < 0.0001 vs. baseline). After meal ingestion, glucose and insulin AUC decreased, whereas the glucagon response increased (all P < 0.001). While oral glucose appearance was unchanged, EGP was increased (median, 40 [IQR, 14] g and 37 [IQR, 11] g vs. 34 [IQR, 11] g, both P < 0.01). Tissue glucose disposal was reduced (median, 75 [IQR, 16] g and 70 [IQR, 21] g vs. 93 [IQR, 18] g, P < 0.0001), due to a decrease in both glucose oxidation and nonoxidative glucose disposal, with a concomitant rise in lipid oxidation after chronic administration (all P < 0.01). β Cell glucose sensitivity increased (median, 55 [IQR, 35] pmol·min⁻¹·m⁻²·mM⁻¹ and 55 [IQR, 39] pmol·min⁻¹·m⁻²·mM⁻¹ vs. 44 [IQR, 32] pmol·min⁻¹·m⁻²·mM⁻¹, P < 0.0001), and insulin sensitivity was improved. Resting energy expenditure rates and those after meal ingestion were unchanged.

Conclusions. In patients with type 2 diabetes, empagliflozin-induced glycosuria improved β cell function and insulin sensitivity, despite the fall in insulin secretion and tissue glucose disposal and the rise in EGP after one dose, thereby lowering fasting and postprandial glycosuria. Chronic dosing shifted substrate utilization from carbohydrate to lipid.

Trial registration. ClinicalTrials.Gov NCT01248364 (EudraCT no. 2010-018708-99).

Funding. This study was funded by Boehringer Ingelheim.
When large amounts of glucose — ranging from 50 to 100 g daily — and the corresponding calorie equivalent (200–400 kcal/d) are pharmacologically forced into urinary excretion, whole-body metabolism must undergo adaptive changes involving glucose fluxes, hormonal responses, fuel selection, and energy expenditure. In the present work, we used empagliflozin, a highly potent and selective SGLT2 inhibitor, to investigate the integrated physiological response to forced glycosuria in patients with type 2 diabetes. By combining a mixed meal with the double-tracer technique, we measured the separate contribution of meal-derived glucose, endogenous glucose production (EGP), and whole-body glucose disposal to plasma glucose concentrations. With the use of indirect calorimetry, we assessed the attendant changes in substrate utilization and energy expenditure. Specifically, we tested the following questions:

(a) whether the glycosuric effect of the inhibitor is maintained over time, (b) whether EGP is altered in response to the glycosuria, (c) how the lower plasma glucose levels affect β-cell function and insulin sensitivity, (d) how tissue substrate utilization adapts to the glucose leak, (e) what is the hormonal background of these responses, and (f) whether the glycosuria-induced energy deficit alters resting energy expenditure and the thermogenic response to feeding.

Results
The final patient population (Figure 1) consisted of overweight/obese patients, mostly with a disease duration longer than 5 years, with preserved renal function and good glycemic control (Table 1). Baseline. During the 3 hours preceding meal ingestion, plasma glucose concentrations decreased by 1.3 ± 0.8 mmol/l (P < 0.0001),...
reflecting progression of the fast; over this time period, the estimated prehepatic insulin/glucagon molar concentration ratio did not change (Table 1). Following the ingestion of the mixed meal, plasma glucose excursions peaked at 30 minutes (by $\sim 5$ mmol/l on average) and did not return to baseline values before 5 hours (Figure 2). Plasma insulin and glucagon concentrations, and their estimated prehepatic molar concentration ratios, rose and fell in a time pattern parallel to that of glycemia (Figure 2 and Table 2). The relationship between insulin secretion rates and concomitant plasma glucose concentrations (i.e., $\beta$ cell glucose sensitivity) was typical of this kind of patients (refs. 19, 20, and Figure 3), the average value (median, 44 [interquartile range {IQR}, 32] pmol/min $^{-1}$•m$^{-2}$•mM$^{-1}$) being reduced by approximately 60% in comparison with that of a historical control group of nondiabetic subjects studied with the exact same protocol (98 pmol/min $^{-1}$•m$^{-2}$•mM$^{-1}$) (19, 20). Fasting insulin secretion rate was within the normal range (21), extrapolating to approximately 27 U/m$^2$ over 24 hours; an average incremental output of 11 U/m$^2$ insulin was used to dispose of the meal. Both potentiation and rate sensitivity were reduced in comparison with those in nondiabetic subjects (1.73 and 1,081 pmol•m$^{-2}$•mM$^{-1}$, respectively) (refs. 19, 20, and Table 3). The meal elicited a robust and protracted GLP-1 response and a large decrement in circulating FFA concentrations (Figure 4).

During the period after meal ingestion, 61 g oral glucose had appeared in the systemic circulation by the end of 5 hours (Table 4), at which time oral glucose was still appearing at a rate of approximately 5 μmol/kg FFM$^{-1}$•min$^{-1}$ (Figure 5A). As glycosuria was negligible (Table 5), body tissue disposal was almost entirely attributable to the sum of oral (61 g) and endogenous glucose (34 g); oxidation and nonoxidative glucose disposal contributed in an approximate ratio of 1:2.5 (Figure 6). Insulin sensitivity — as the ratio of mean glucose metabolic clearance rate (MCR) to mean insulin concentration during meal absorption — was severely impaired, its value being only one-third of that of lean nondiabetic subjects (24 ml•kg$^{-1}$•min$^{-1}$•nM$^{-1}$) fed the same meal (20). Meal-induced thermogenesis averaged 12% (Figure 7).

Acute study. After a single first dose of 25 mg empagliflozin, patients excreted an average of 8 g glucose into the urine over the

### Table 2
Glucose, hormones, and FFA during the meal$^a$

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Acute</th>
<th>Chronic</th>
<th>$P_B$</th>
<th>$P_C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_G$ (gd$^{-1}$•h) [IQR]</td>
<td>57 [16]</td>
<td>51 [11]</td>
<td>51 [10]</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AUC$_I$ (nmol$^{-1}$•h) [IQR]</td>
<td>93 [65]</td>
<td>80 [59]</td>
<td>76 [59]</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AUC$_{GIG}$ (nmol$^{-1}$•h)</td>
<td>1.07 ± 0.32</td>
<td>1.33 ± 0.42</td>
<td>1.15 ± 0.36</td>
<td>&lt;0.0001</td>
<td>0.0005</td>
</tr>
<tr>
<td>Meal I/Glg ratio (mol/mol) [IQR]</td>
<td>29 [19]</td>
<td>22 [17]</td>
<td>24 [19]</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AUC$_{GLP-1}$ (nmol$^{-1}$•h)</td>
<td>8.7 ± 4.1</td>
<td>10.4 ± 3.9</td>
<td>9.2 ± 3.9</td>
<td>0.0013</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$_{GIP}$ (nmol$^{-1}$•h)</td>
<td>38.3 ± 38.4</td>
<td>39.8 ± 43.7</td>
<td>36.5 ± 43.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>AUC$_{FAA}$ (mEq$^{-1}$•h)</td>
<td>68 ± 23</td>
<td>86 ± 29</td>
<td>94 ± 33</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin MCR (l•min$^{-1}$•m$^2$)</td>
<td>1.20 ± 0.42</td>
<td>1.32 ± 0.46</td>
<td>1.43 ± 0.53</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$^a$Data are mean ± SD or median [IQR] for normally or nonnormally distributed parameters; correspondingly, $P$ values are from paired $t$ test or Wilcoxon signed-rank testing ($P_B$ = acute vs. baseline, $P_C$ = chronic vs. baseline). G, glucose; I, insulin; Glg, glucagon; I/Glg ratio, estimated prehepatic insulin-to-glucagon molar concentration ratio.
subsequent 3 hours of fasting and 29 g over the 5-hour period after meal ingestion (Figure 8 and Table 5). Fasting plasma glucose levels (before drug administration) were similar to the corresponding values of the baseline study, but at time 0 — i.e., after 3 hours of fasting — glycemia had dropped by 2.2 ± 0.9 mmol/l, a significantly (P < 0.0001) greater change than that seen in the baseline study (Δ = 0.9 ± 0.7 mmol/l) (Table 1). As plasma insulin decreased more than plasma glucagon, their prehepatic molar concentration ratio at time 0 was significantly decreased as compared with that at baseline (from 9 [IQR, 5] to 7 [IQR, 4] mol/mol, P < 0.0001).

Over the postprandial period, the area under the glucose curve was significantly reduced (by 12%), as were the plasma insulin and total insulin secretory responses (Tables 2 and 3). In contrast, the plasma glucagon response was increased (Figure 4), whereby the estimated prehepatic insulin-to-glucagon ratio was decreased, as compared with that at baseline (Figure 3). Fasting EGP was increased by approximately 30%, and EGP was also greater throughout meal absorption (Table 4 and Figure 5B), the difference from the baseline study being greater than 0 at all time points (Figure 5C). Whereas appearance of oral glucose was similar to that at baseline, tissue glucose disposal (TGD) was 20% reduced — virtually entirely due to a fall in nonoxidative glucose disposal (Figure 6) — despite a significant improvement in insulin sensitivity (Table 4). Neither lipid nor protein oxidation was different from those at baseline. Resting energy expenditure and meal-induced thermogenesis were superimposable on the baseline values. With regard to β cell function, glucose sensitivity was improved and total insulin output was decreased, while potentiation and rate sensitivity did not change (Table 3). The GLP-1 response to the meal was enhanced, while meal-induced FFA suppression was significantly blunted (Table 2 and Figure 4).

Chronic study. After 4 weeks of treatment, 25 mg empagliflozin caused fasting and after meal glycemia, which were similar in quantity and time course to the acute study (Figure 7 and Table 5). As compared to the baseline study, HbA1c, fasting, and mean glucose levels after meal ingestion were all significantly decreased (Tables 1 and 2). The fasting plasma insulin concentration and the insulin response to the meal were reduced, whereas the glucagon response was still increased, though somewhat less than during the acute study. As a consequence, the estimated prehepatic insulin-to-glucagon ratio was reduced as compared with that at baseline. As was the case in the acute study, both fasting and EGP after meal ingestion were higher than in the baseline study, although differences after meal ingestion were attenuated (Figure 5C). Appearance of oral glucose was comparable to that at baseline and the acute study. TGD was decreased in the fasting state as well as throughout the meal, with both glucose oxidation and nonoxidative glucose disposal contributing to the decrease (Table 4). The decrease in glucose oxidation was matched by an increase in lipid oxidation, with no change in protein oxidation or meal-related energy expenditure (Table 5 and Figures 6 and 7).

Insulin sensitivity was numerically — but not statistically significantly — improved as compared to baseline, whereas β cell glucose sensitivity was enhanced to a similar extent as in the acute study; neither potentiation nor rate sensitivity was different.

The GLP-1 response still appeared somewhat enhanced (though not significantly), and FFA suppression was still impaired. It is of note that insulin clearance was significantly increased following both acute and chronic empagliflozin administration (Table 2).

Table 3

<table>
<thead>
<tr>
<th>β Cell function parameters&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Baseline</th>
<th>Acute</th>
<th>Chronic</th>
<th>P&lt;sup&gt;B&lt;/sup&gt;</th>
<th>P&lt;sup&gt;C&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting ISR (pmol·min&lt;sup&gt;-1&lt;/sup&gt;·m&lt;sup&gt;-2&lt;/sup&gt;) [IQR]</td>
<td>112 [52]</td>
<td>113 [44]</td>
<td>102 [45]</td>
<td>NS</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total IS (nmol·m&lt;sup&gt;-2&lt;/sup&gt;) [IQR]</td>
<td>102 [46]</td>
<td>95 [47]</td>
<td>101 [48]</td>
<td>0.0025</td>
<td>NS</td>
</tr>
<tr>
<td>β-GS (pmol·min&lt;sup&gt;-1&lt;/sup&gt;·m&lt;sup&gt;-2&lt;/sup&gt;·mM&lt;sup&gt;-1&lt;/sup&gt;) [IQR]</td>
<td>44 [32]</td>
<td>55 [35]</td>
<td>55 [39]</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
</tr>
<tr>
<td>Potentiation factor (ratio) [IQR]</td>
<td>1.17 [0.34]</td>
<td>1.12 [0.34]</td>
<td>1.07 [0.22]</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Rate sensitivity (pmol·min&lt;sup&gt;-1&lt;/sup&gt;·mM&lt;sup&gt;-1&lt;/sup&gt;) [IQR]</td>
<td>164 [607]</td>
<td>175 [430]</td>
<td>220 [365]</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are median [IQR]; correspondingly, P values are from Wilcoxon signed-rank testing (P<sup>B</sup> = acute vs. baseline, P<sup>C</sup> = chronic vs. baseline). ISR, insulin secretion rate; IS, insulin secretion; β-GS, β cell glucose sensitivity.
Discussion
The major new observation of this study is that enhancing glycosuria with a single dose of the SGLT2-inhibitor, empagliflozin, led to a reduction in insulin secretion and TGD and a rise in EGP. Despite the fall in insulin secretion, $\beta$ cell function was improved; in the face of lower insulin concentrations, insulin sensitivity of tissue glucose uptake also was improved. Because empagliflozin does not directly affect either $\beta$ cell function or tissue glucose utilization, the current results demonstrate that lowering glucose levels can rapidly, though partially, relieve the glucose toxicity of patients with type 2 diabetes, confirming previous findings in rodents (4).

In this cohort of patients with well-controlled type 2 diabetes with preserved renal function, a single 25-mg dose of empagliflozin — administered after an overnight fast — induced substantial glycosuria (8 g) over the subsequent 3 hours of fasting. This elicited a rise in EGP that averaged 7 g over those 3 hours, thereby exactly balancing the glucose lost through the urine and accounting for the reduction in plasma glucose concentrations. It can be calculated that, had the rise in EGP not occurred, the same glycosuria would have caused fasting glycemia to fall to 4.7 mmol/l instead of the observed 6.7 mmol/l, i.e., to normoglycemic levels. Signals for this immediate EGP response presumably were the changes in glycemia, the drop in the estimated prehepatic insulin-to-glucagon ratio (from 9 [IQR, 5] to 7 [IQR, 4] mol/mol), the prompt rise in circulating FFA levels (which may enhance gluconeogenesis; refs. 22, 23), and, possibly, yet unidentified factors.

In response to the meal, empagliflozin caused marked glycosuria (29 g over 5 hours), which led to a significant decrease in glycemic excursions but also to a reduction in the insulin secretory response and an augmentation of the glucagon response, such that the prehepatic insulin-to-glucagon concentration ratio decreased by 25%. While the lower insulin response is easily explained by the lower glucose levels — which remain the main driver of insulin secretion under all circumstances — multiple factors could contribute to the relative hyperglucagonemia. Both hyperglycemia — by a direct action on the $\alpha$ cell — and increased insulin release — through a paracrine mechanism (24) — restrain glucagon release, but we cannot rule out the participation of other factors. The mechanism of the heightened GLP-1 response remains undetermined, since acute SGLT2 inhibition was not associated with changes in the appearance of oral glucose that would indicate changes in gastric emptying (such as occurs, for example, following gastric bypass surgery; ref. 20). Conversely, there is good evidence that GLP-1 changes within the normal range play no role in the regulation of gastric emptying (25). It is of interest that the greater GLP-1 response, though small ($\sim 20\%$), should have suppressed glucagon release. With regard to this, growing evidence suggests colocalization and cosecretion of multiple hormones by intestinal cells (26–28).

### Table 4
Glucose fluxes in the fasting state and during the meal (5 hours)$^A$

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Acute</th>
<th>Chronic</th>
<th>$P^B$</th>
<th>$P^C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting EGP (μmol·kg$\text{FFM}^{-1}·\text{min}^{-1}$) [IQR]</td>
<td>13.8 [5.2]</td>
<td>17.6 [4.8]</td>
<td>17.5 [4.1]</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Fasting TGD (μmol·kg$\text{FFM}^{-1}·\text{min}^{-1}$) [IQR]</td>
<td>14.9 [5.4]</td>
<td>14.8 [5.2]</td>
<td>12.9 [4.5]</td>
<td>NS</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Ra$\text{T AUC}$ (g) [IQR]</td>
<td>95 [15]</td>
<td>102 [12]</td>
<td>98 [12]</td>
<td>$&lt;0.0001$</td>
<td>0.0033</td>
</tr>
<tr>
<td>Rd$\text{AUC}$ (g) [IQR]</td>
<td>95 [18]</td>
<td>102 [16]</td>
<td>99 [13]</td>
<td>$&lt;0.0001$</td>
<td>NS</td>
</tr>
<tr>
<td>TGD$\text{AUC}$ (g) [IQR]</td>
<td>93 [18]</td>
<td>75 [16]</td>
<td>70 [21]</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Insulin sensitivity (ml·kg$\text{FFM}^{-1}·\text{min}^{-1}·\text{nM}^{-1}$)</td>
<td>8.2 [5.8]</td>
<td>9.1 [6.7]</td>
<td>8.6 [8.0]</td>
<td>0.0226</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^A$Data are median [IQR]; correspondingly, $P$ values are from Wilcoxon signed-rank testing ($P^B =$ acute vs. baseline, $P^C =$ chronic vs. baseline). Ra$T$, rate of total glucose appearance.
The makeover of the hormonal milieu following meal-related glycosuria — less insulin, more glucagon, and more GLP-1 — had important metabolic consequences. First, suppression of EGP during meal absorption was significantly blunted, amounting to an extra approximately 6 g of glucose added to the systemic circulation as compared with the control study. Second, the concomitant fall in plasma glucose and insulin concentrations caused a drop in TGD of approximately 18 g over 5 hours, almost entirely accounted for by a decrease in nonoxidative glucose utilization. It can therefore be calculated that, had EGP and TGD remained unaltered in the face of glycosuria, glucose levels after meal ingestion would have decreased by approximately 50% instead of the observed 12%. However, the efficiency of insulin-mediated glucose disposal, as estimated by the ratio of metabolic glucose clearance to insulin levels, was significantly, though only marginally, improved. Third, when viewed in the context of the prevailing glucose levels, insulin secretion was enhanced by approximately 25%. Both the increased GLP-1 response and the reduction of hyperglycemia were likely mechanisms underlying this acute improvement in β cell glucose sensitivity. There was, however, no significant change in either potentiation or rate sensitivity, indicating an overall limited impact on β cell function. Finally, it is of note that the acute deficit of glucose as a substrate did not translate into a higher oxidative use of either lipid or protein substrates, as the energy derived from glucose oxidation was maintained. Thus, there was only a minimal decrement in fasting energy expenditure, without any impairment of meal-induced thermogenesis.

After chronic treatment, the effect of a morning dose of empagliflozin on fasting and glycosuria after meal ingestion was superimposable on that seen following the first dose. This is explained by the fact that the renal glucose threshold, which at baseline marked the appearance of glycosuria at plasma glucose concentrations above approximately 11.1 mmol/l, was shifted to levels well within the normoglycemic range (Figure 8B). This is the expected consequence of a partial inhibition of tubular glucose reabsorption, extending throughout the range of plasma and intraluminal glucose concentrations, induced by competitive binding of empagliflozin to tubular SGLT2 (29). This finding has clinical relevance,

<table>
<thead>
<tr>
<th>Table 5</th>
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</thead>
<tbody>
<tr>
<td><strong>UGE and substrate oxidation rates</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>Fasting urine output (l) [IQR]</td>
</tr>
<tr>
<td>Fasting UGE&lt;sub&gt;Ac&lt;/sub&gt; (g) [IQR]</td>
</tr>
<tr>
<td>Meal urine output (l) [IQR]</td>
</tr>
<tr>
<td>Meal UGE&lt;sub&gt;Ac&lt;/sub&gt; (g) [IQR]</td>
</tr>
<tr>
<td>Meal NPRQ [IQR]</td>
</tr>
<tr>
<td>GOx&lt;sub&gt;Ac&lt;/sub&gt; (g) [IQR]</td>
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<tr>
<td>NOGD&lt;sub&gt;Ac&lt;/sub&gt; (g) [IQR]</td>
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<tr>
<td>Meal LOX&lt;sub&gt;Ac&lt;/sub&gt; (g) [IQR]</td>
</tr>
<tr>
<td>Fast EE (kJ/min) [IQR]</td>
</tr>
<tr>
<td>Meal EE (kJ/min) [IQR]</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are median [IQR]; correspondingly, P values are from Wilcoxon signed-rank testing (P<sub>b</sub> = acute vs. baseline, P<sub>c</sub> = chronic vs. baseline). NPRQ, nonprotein respiratory quotient; GOx, glucose oxidation; NOGD, nonoxidative glucose disposal; LOX, lipid oxidation; POX, protein oxidation; EE, energy expenditure.
in that little weakening of the primary effect of SGLT2 inhibition is to be expected as plasma glucose levels decrease with chronic treatment. Also, both acutely and chronically, the observed glycosuria amounted to a deficit of approximately 20% of the calorie content of the meal.

After 4 weeks of treatment, both HbA1c and fasting glucose levels declined significantly, and postprandial glucose exposure was reduced to a similar extent as with single-dose administration in concomitance with even lower peripheral plasma insulin levels (Table 2). The physiological adaptation to chronic drug-induced glycosuria was qualitatively similar to that seen after the first empagliflozin administration, with a few differences. While fasting EGP was increased to a similar extent, the excess EGP during the meal was somewhat blunted, amounting to only 3 g over 5 hours. Correspondingly, the glucagon response also was attenuated, and the GLP-1 response was no longer different from baseline. The improvement in β cell function was similar to that in the acute study, and estimated insulin sensitivity was at least maintained as compared to baseline. The main difference consisted of a larger decrement in TGD — now affecting both nonoxidative glucose disposal (as in the acute study) and glucose oxidation — as a joint result of prolonged reductions in insulin output and glucose levels (30). As a consequence, energy expenditure was maintained at the cost of a rise in lipid oxidation, which was paralleled (and supported) by higher FFA concentrations (AUC\textsubscript{FFA}: 94 ± 33 vs. 86 ± 29 mEq•l\textsuperscript{–1}•h, \(P < 0.01\)).

In summary, pharmacological blockade of renal glucose reabsorption does reduce fasting and postprandial glycemia both acutely and chronically without causing hypoglycemia, confirming the potential use of empagliflozin as a glucose-lowering agent in patients with type 2 diabetes (18). As expected from studies in experimental diabetes (4), both β cell function and insulin sensitivity are improved, though the full extent of such changes probably requires longer drug exposure with additional relief of glucotoxicity. Pharmacologically induced glycosuria does not alter the appearance of oral glucose (or, presumably, gastric emptying) but results in a compensatory increase in endogenous glucose release and lower tissue glucose uptake as a consequence of lower glucose and insulin levels and higher glucagon levels. In the longer term, the attendant deficit of glucose oxidation is made up for by extra lipid oxidation, thereby maintaining energy balance. Whereas enhanced oxidative lipid utilization — along with the calorie deficit induced by urinary glucose loss — is expected to eventually lead to reduced fat mass (31), it is relevant to observe that treatment with empagliflozin does not result in reduced rates of energy expenditure or a blunted thermogenic effect of feeding. Therefore, the observation in longer-term clinical trials that weight loss is less than predicted by glycosuria suggests that this form of treatment stimulates calorie intake (32).

One limitation of this study is the absence of a control group made up of nondiabetic subjects or patients with type 2 diabetes matched for changes in glucose control or energy deficit. However, the current observations were made in patients with type 2 diabetes with a rather typical clinical phenotype, in whom SGLT2 inhibition, especially as an add on to metformin, would presumably represent a viable approach to achieving target glycemic control.
12 weeks) were recruited into the study (Figure 1). Inclusion criteria were sex, age >18 years, BMI between 20 and 40 kg/m², and HbA1c between 47.5 and 14.1 mmol/mol (6.5%–10.5%). Exclusion criteria were history of malignancy in the last 5 years, marked cardiovascular disorder within the last 6 months, pregnancy or the expectation of conception within the study duration, bariatric surgery within the past 2 years, treatment with antiobesity drugs in the last 3 months, impaired renal function defined as an EGFR <60 ml/min 1.73 m⁻², neurogenic bladder disorders, ALT and AST >3.0xULN, changes in thyroid hormone dosage within 6 weeks, presence of any other endocrine disease except type 2 diabetes, and alcohol or drug abuse.

**Study design and protocol.** Patients with type 2 diabetes received empagliflozin in an open-label design; each subject underwent 3 studies: baseline, acute (first single dose of 25 mg empagliflozin), and chronic (28 days of 25 mg/d empagliflozin). Each study consisted of a 5-hour meal tolerance test following a 3-hour basal period combined with a double-tracer technique, as described previously (33). A primed-constant infusion of [6,6-²H₂]-glucose (0.28 μmol/min ¹kg⁻¹; prime 28 μmol/kg⁻¹x5), where x stands for fasting plasma glucose in mmol/l, was administered throughout the test, starting at time point –180 minutes. At time 0, subjects ingested the test, starting at time point –180 minutes. At time 0, subjects ingested a meal consisting of 1 egg, 50 g parmesan cheese, 50 g free glucose, because only this part of the ingested carbohydrate could be expressed as grams per hour. Plots represent mean ± SD. (B) The glucose excretion data in A are plotted against the corresponding mean plasma glucose concentration during the corresponding periods of urine collection (n = 66) for the baseline study (blue lines) and the 2 studies after dosing (n = 132); the latter were combined because they were fully overlapping (red lines). The blue lines are the quadratic polynomial fit and its 95% confidence intervals for the baseline data, and the red lines are the linear fit and its 95% confidence interval for the empagliflozin studies. The top x axis indicates HbA1c levels corresponding to the mean plasma glucose concentrations on the bottom x axis, derived by linear correlation of the entire data set.

**Figure 8**
UGE. (A) Time course of UGE during the fasting period (–180 to 0 minutes) and the period after meal ingestion (0 to 300 minutes) in patients with type 2 diabetes during the baseline study, after a single dose (acute study), and after 28 days of empagliflozin dosing (chronic study). Data were averaged over 30-minute time intervals and expressed as grams per hour. Plots represent mean ± SD. (B) The glucose excretion data in A are plotted against the corresponding mean plasma glucose concentration during the corresponding periods of urine collection (n = 66) for the baseline study (blue lines) and the 2 studies after dosing (n = 132); the latter were combined because they were fully overlapping (red lines). The blue lines are the quadratic polynomial fit and its 95% confidence intervals for the baseline data, and the red lines are the linear fit and its 95% confidence interval for the empagliflozin studies. The top x axis indicates HbA1c levels corresponding to the mean plasma glucose concentrations on the bottom x axis, derived by linear correlation of the entire data set.
prolonged suppression of EGP, e.g., Figure 5 in ref. 20). This choice is justi-

fied as follows: (a) the meal with bread totals 710 kcal and is closer to a real
meal; (b) the same meal was given basally, acutely, and chronically; (c) RoA
was virtually superimposable among the 3 studies (Figure 5A), suggesting
no difference in gastric emptying and, by extension, in the digestion of the
bread; and (d) the presence of bread obviously does not affect fasting EGP,
which is the condition under which the difference in EGP from baseline is
highest (Figure SC).

Whole-body glucose utilization (or rate of disappearance; μmolmin⁻¹
kgFFM⁻¹) was obtained as the product of clearance and total glucose con-
centration. For ease of comparison with common units of urinary glucose
excretion (UGE), time-integrated glucose fluxes were expressed in grams.

The prehepatic insulin-to-glucagon molar concentration ratio (I/GL)
was estimated by the following formula: \((\text{ISR}(t)/\text{hPF} + [\text{I}(t)])/[\text{GL}(t)) \times
(1 + \text{MCRGlg}/\text{hPF})\), where ISR(\(t\)) is the insulin secretion rate at time \(t\), hPF
is hepatic plasma flow; [\(\text{I}(t)\)] and [\(\text{GL}(t)\)] are the measured (peripheral) plasma
concentrations of insulin and glucagon at time \(t\), respectively; and \(\text{MCRGlg}\)
is the MCR of glucagon. hPF was estimated by multiplying the cardiac index
(3.2 l/min⁻¹·m⁻²) (39) by a plasma-to-blood ratio of 0.6 and by assuming
that hepatic blood flow is 30% of cardiac index (0.576 l/min⁻¹·m⁻²) (40). \(\text{MCRGlg}\)
was taken to be 0.537 l/min⁻¹·m⁻² (41).

Peripheral insulin sensitivity was estimated as the ratio of the average
insulin MCR during the 5 hours after meal ingestion to the corresponding
mean plasma insulin concentration. UGE was calculated as the product
of urine volume and urine glucose concentration and was subtracted from
the rate of total glucose disappearance (Rd) to obtain TGD rate. MCR
of glucose (MCR, mmoles⁻¹·kgFFM⁻¹) was calculated as the ratio of TGD to
mean plasma glucose concentration over corresponding period of time. Glucose
oxidation rate, lipid oxidation rate, protein oxidation rate, and energy
expenditure were obtained from indirect calorimetry measurements,
next to g, and, respectively, to allow a rapid comparison with UGE, which is
often more expressed in weight (g) rather than molar units (mmol).

\(\beta\) Cell function modeling. The model used to reconstruct insulin secre-
tion and its control by glucose has been previously described (43). In brief,
the model consists of three blocks: (a) a model for fitting the glucose concentration profile, the purpose of which is to smooth and
interpolate plasma glucose concentrations; (b) a model describing the dependence of insulin (or C-peptide) secretion on glucose concentra-
tion; and (c) a model of a C-peptide kinetics, i.e., the 2-exponential model


disposition of insulin secretion as glucose levels rise. Total insulin secretion is the
sum of the two components described above and was calculated every
10 minutes for the whole 5-hour period.

Insulin MCR was calculated as the ratio of total insulin secretion (as
reconstructed from C-peptide concentrations) to the insulin AUC for cor-
responding time intervals (45, 46).

Statistics. Data are given as mean ± SD or median and IQR for normally
or nonnormally distributed variables, respectively. Acute and chronic treat-
ment responses were analyzed by paired \(t\) test or Wilcoxon signed-rank test,
depending on the underlying data distribution. \(P\) values were Bonferroni
adjusted for double comparison. A 2-tailed \(P\) value of equal to or less than
0.05 was considered statistically significant. All analyses were carried out
using JMP 7.0.

Study approval. The study was carried out at two sites (University of
Pisa School of Medicine and Profil) and according to the Declaration of
Helsinki and the International Conference on Harmonization Good
Clinical Practice principles. The protocol was approved by the Institu-
tional Review Board at each participating site. All participants provided
informed written consent.

Acknowledgments

This study was presented at the 73rd Scientific Sessions of the
American Diabetes Association, Chicago, Illinois, USA, 21–25 June
2013 (abstract LB#71). The authors thank the patients and staff
who participated in this study.

Received for publication August 5, 2013, and accepted in revised
form November 14, 2013.

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The Journal of Clinical Investigation
http://www.jci.org Volume 124 Number 2 February 2014
507