SGLT2 selective inhibitor ipragliflozin reduces body fat mass by increasing fatty acid oxidation in high-fat diet-induced obese rats

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Abstract

Ipragliflozin is a novel and selective sodium-glucose cotransporter 2 (SGLT2) inhibitor that induces sustained increases in urinary glucose excretion by inhibiting renal glucose reabsorption and thereby exerting a subsequent antihyperglycemic effect. Here, we examined the effect of ipragliflozin on body weight in high-fat diet-induced (HFD) obese rats. Treatment of ipragliflozin (10 mg/kg once daily) reduced body weight despite a slight increase in food intake. Dual-energy X-ray absorptiometry and computed tomography demonstrated that the reduction in body weight was accompanied by reduced visceral and subcutaneous fat masses but not lean mass or bone mineral content. Analysis of plasma and urinary parameters suggested the possibility that ipragliflozin enhanced lipolysis and fatty acid oxidation, and indirect calorimetry showed that ipragliflozin decreased the heat production rate from glucose but increased the rate from fat and lowered the respiratory exchange ratio. In conclusion, these data demonstrate that ipragliflozin-induced urinary glucose excretion specifically reduces fat mass with steady calorie loss by promoting the use of fatty acids instead of glucose as an energy source in HFD rats. By
improving hyperglycemia and promoting weight reduction, ipragliflozin may prove useful in treating type 2 diabetes in obese individuals.

**Keywords** Ipragliflozin; Sodium-glucose cotransporter 2; Urinary glucose excretion; Diabetes; Obesity; High-fat diet

1. **Introduction**

The global epidemic of obesity largely explains the dramatic increase in the incidence and prevalence of type 2 diabetes over the past 20 years (Eckel et al., 2011; Despres, 2012). Recent studies have identified links between obesity and type 2 diabetes that include proinflammatory cytokines, insulin resistance, deranged fatty acid metabolism, and cellular processes such as mitochondrial dysfunction and endoplasmic reticulum stress (Unger, 1995; Trayhurn et al., 2011).

Although improvement in glycemic control while minimizing the risk of weight gain or promoting weight reduction is widely understood to be important in the management of type 2 diabetes, the impact of pharmacologically-induced improvements varies by individual drug. Most glucose-lowering medications are associated with effects of weight gain (insulin, sulfonylureas, glinides, and thiazolidinediones) or weight maintenance (metformin, α-glucosidase inhibitors, DPP-4 inhibitors), with only GLP-1 analogues having been reported to promote weight loss (Eckel et al., 2011). In contrast, existing anti-obesity agents, which target the control of appetite, reduce body weight in overweight and obese patients but have limited efficacy on hyperglycemia and in
fact have been found to exert severe adverse effects, including cardiovascular disease risk and neuropsychiatric effects (Colagiuri, 2010). As such, additional agents that not only improve hyperglycemia but also promote weight loss are needed for the successful management of type 2 diabetes in overweight and obese patients.

Sodium-glucose cotransporters (SGLTs) are members of the solute carrier family 5A (SLC5A) and specialize in the cotransport of sodium and glucose across different cell types (Wright et al., 2007). Among these, SGLT2 is a low-affinity, high-capacity transporter exclusively expressed in the renal proximal tubules (Wells et al., 1992). SGLT2 gene mutations have been associated with familial renal glucosuria (van den Heuvel et al., 2002), and SGLT2 deficient mice have shown higher urinary glucose excretion than wild type mice (Vallon et al., 2011; Jurczak et al., 2011), demonstrating that SGLT2 plays an important role in renal glucose reabsorption both in humans and rodents. Further, inhibition of renal reabsorption has been found effective in reducing the renal threshold for glucose, thus allowing for the excretion of glucose into the urine.

We recently identified the novel orally active and selective SGLT2 inhibitor ipragliflozin (ASP1941), which is currently being clinically developed for use in treating patients with type 2 diabetes (Imamura et al., 2012; Tahara et al., 2012a, 2012b, 2013). In several clinical trials, ipragliflozin treatment not only improves hyperglycemia but also reduces body weight in type 2 diabetic patients (Schwartz et al., 2011; Wilding et al., 2013; Fonseca et al., 2013; Kashiwagi et al., 2010, 2011). Body weight reductions have also been observed with other SGLT2 inhibitors (Ferrannini et al., 2010, 2013, Zhang et al., 2010; Bolinder et al., 2012, Inagaki et al., 2013).
Although this weight reduction is considered an effect of either or both steady caloric loss or water loss through increased glucosuria, changes in body composition and the mechanisms responsible have not been elucidated. Here, we investigated the underlying components and mechanisms of this weight loss during ipragliflozin treatment in high-fat diet (HFD)-induced obese rats.

2. Materials and methods

2.1. Materials

Ipragliflozin (ASP1941; (1S)-1,5-anhydro-1-C-{3-[1-benzothiophen-2-yl]methyl}-4-fluorophenyl}-D-glucitol compound with L-proline [1:1] (purity>99%) was synthesized at Astellas Pharma Inc. (Ibaraki, Japan) (Imamura et al., 2012). This was suspended in 0.5% methylcellulose solution, and administered orally. All doses are expressed as the free base form.

2.2. Animals

Male Sprague-Dawley rats aged 6 weeks were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) and used at 7 weeks of age. All rats had free access to food and water for the duration of the study and were housed under conventional conditions with controlled temperature, humidity, and light (12-h light-dark cycle). Rats were fed either a normal chow diet (total caloric energy value = 3.45 kcal/g; CE-2; CLEA Japan, Inc., Tokyo, Japan) or a high-fat diet (consisting of 45% fat, 35% carbohydrate, and 20% protein, total caloric energy value = 4.73 kcal/g)
kcal/g; D12451; Research Diet Inc., New Brunswick, NJ, USA). After 3 weeks, the high-fat diet-fed rats were allocated into 2 groups such that each group had similar body weights and plasma glucose and insulin levels. Drugs were given once daily in the evening. Animals were handled and cared for in accordance with the Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the Animal Ethical Committee of Astellas Pharma Inc.

2.3. Tissue weight, body weight, food intake, plasma, urinary, and hepatic parameters

After grouping, HFD rats were given vehicle or ipragliflozin (10 mg/kg) for 4 weeks. Normal diet-fed (ND) rats were given vehicle. Body weight and food intake were measured every week. In the 3rd week of drug treatment, blood samples were collected 14 h after final dosing from the tail vein under non-fasting or 14-h-fasted conditions. Plasma glucose, non-esterified fatty acid (NEFA), and triglyceride levels were determined using the Glucose CII-Test WAKO, NEFA C-Test WAKO, triglyceride E-Test WAKO, respectively (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma creatinine levels were measured using CRE-EN Kainos (Kainos Laboratories, Tokyo, Japan). Plasma total cholesterol and high-density lipoprotein (HDL)-cholesterol levels were determined using Determiner L TCII and HDL-C, respectively (Kyowa Medex Co., Tokyo, Japan). Plasma insulin and leptin levels were determined using the rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Gunma, Japan) and Leptin Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA), respectively. The level of plasma 3-hydroxybutyrate was determined using Ketorex “Sanwa” (Sanwa Kagaku Kenkyuusho Co. Ltd., Nagoya, Japan).
Spontaneously voided urine was collected for 24 h using metabolic cages under non-fasting conditions. After the urine volume had been measured, glucose and 3-hydroxybutyrate concentrations in the urine were measured as described above. The urinary excretion of glucose and 3-hydroxybutyrate was calculated as the product of the urine concentration and the urine volume. In the fourth week of treatment, rats were sacrificed under anesthesia, and epididymal, retroperitoneal, and mesenteric fat, liver, and kidney were removed and tissue weights measured.

2.4. Imaging analysis

After grouping, HFD rats were given vehicle or ipragliflozin (10 mg/kg) for 4 weeks. ND rats were given vehicle. Whole-body compositions were measured using dual-energy X-ray absorptiometry (DEXA) (QDR 2000; Hologic, Waltham, MA, USA) and the accompanying small animal software package (version 7.10). This software provides data on total body fat and lean soft tissue masses as well as bone mineral content (BMC), and its application in extremely young animals has been validated (Engelbregt et al., 1999). Before each series of measurements, a tissue calibration scan was performed using the Hologic phantom. Each rat was placed on a Plexiglas platform in a prone position, and BMC, bone mineral density, and whole body weight (BMC + lean body mass + fat mass) were measured. Abdominal adipose tissue mass was also analyzed using small-animal computed tomography (CT), as described previously (Luu et al., 2009). Briefly, animals were placed prone in the appropriate holders, and CT scans were performed using R_mCT (Rigaku Corporation, Tokyo, Japan) with 90 kV tube voltage and a constant 200 mA current for 17 seconds.
while animals were under 2% isoflurane anesthesia. Adipose tissue mass in the abdominal region between the proximal end of the 1st lumbar vertebra and the 1st sacral vertebra was analyzed. The abdominal muscular wall was used to separate visceral adipose tissue from subcutaneous adipose tissue using CT Axial Metabolic Analysis Version 1.41 (Rigaku Corporation). For the abdominal region of each animal, total adipose mass was divided into visceral or subcutaneous. Visceral and subcutaneous adipose mass are expressed as a percentage of total abdominal body mass.

2.5. Indirect calorimetry

After grouping, HFD rats were given vehicle or ipragliflozin (10 mg/kg) for 3 weeks. In the 3rd week of treatment, rats were placed into metabolic chambers with free access to water and food. Indirect calorimetry was measured during dark phase (from 19:00 to 07:00) using an open-circuit indirect calorimetry system (Oxymax®; Columbus Instruments, OH, USA). Oxygen consumption and carbon dioxide production were determined for 1 minute every 15 minutes in each cage. Total energy expenditure was calculated in accordance with Livesey’s method (Livesey and Elia, 1988). In each animal, spontaneously voided urine was collected for determination of urinary nitrogen levels. Urine urea nitrogen was measured by an enzymatic colorimetric method (BUN Kainos; Kainos laboratories, Tokyo, Japan) and extrapolated to total urine nitrogen by multiplying by 1.25. Twelve-hour urine nitrogen was then calculated to obtain 12-h non-protein respiratory quotient in the dark phase.
2.6. **Statistical analysis**

Experimental results are expressed as mean ± standard error of the mean (S.E.M.). Student’s *t*-test was used to analyze differences between two groups. *P*<0.05 was defined as significant. Statistical and data analysis were conducted using the SAS 8.2 software package (SAS Institute Japan, Ltd., Tokyo, Japan).

### 3. Results

#### 3.1. Effects of ipragliflozin on body weight, fat mass, and whole-body composition

Given that a preliminary dose-finding study found that treatment of ipragliflozin to HFD rats for 4 weeks significantly suppressed an increase in body weight at a dose of 10 mg/kg (Supplementary Figure 1), we performed a detailed analysis of this dose regimen. HFD rats had significantly higher body weights than normal diet-fed (ND) rats throughout the experimental period (Figure 1A), but treatment with ipragliflozin significantly suppressed increases in body weight from 2 weeks after administration, and body weights after 4 weeks’ treatment of vehicle and ipragliflozin were 518.2 ± 9.4 g and 456.4 ± 15.2 g, respectively. The average daily food intake was slightly greater in ipragliflozin-treated rats than in vehicle-treated rats (Figure 1B, 91.0 and 94.9 kcal/day in vehicle and ipragliflozin groups, respectively).

Mean visceral (epididymal, retroperitoneal, and mesenteric) fat weight in HFD rats was significantly higher than in ND rats (Figure 2A-C), but no significant differences were noted in liver or kidney weight between diet groups (Figure 2D, E). In the ipragliflozin-treated group, mean
retroperitoneal and mesenteric fat weights were significantly lower than those in vehicle-treated animals (Figure 2B, C), and the epididymal fat weight tended to be lower as well (Figure 2A, \( P=0.055 \)). In contrast, no significant differences were noted in liver or kidney weight between the vehicle- and the ipragliflozin-treated groups (Figure 2D, E).

Effects of ipragliflozin on body composition were estimated using dual-energy X-ray absorptiometry (DEXA) (Figure 3). All body component values, including fat and lean body masses, and bone mineral content were significantly higher in HFD rats than in ND ones (Figure 3B-D). While whole-body fat mass in the ipragliflozin-treated group tended to be less than that in the vehicle group (\( P=0.089 \)) (Figure 3B), ipragliflozin did not show any impact on lean body mass or bone mineral content (Figure 3C, D).

Visceral and subcutaneous fat mass in the abdominal area were also confirmed via computed tomography (CT) analysis (Figure 4). Whole abdominal fat, visceral fat, and subcutaneous fat masses in HFD rats were significantly higher than those in ND rats (Figure 4B-D). In the ipragliflozin-treated group, the whole abdominal fat and visceral fat masses were significantly lower than those of the vehicle-treated group, and subcutaneous fat mass tended to be lower in ipragliflozin-treated animals as well (\( P=0.11 \)) (Figure 4B-D).

3.2. Effects of ipragliflozin on plasma and urinary parameters

Non-fasting plasma, fasting plasma, and urinary parameters are shown in Figures 5, 6, and Table 1. HFD rats did not exhibit an increase in plasma glucose levels or urinary glucose excretion but did
show a significant increase in non-fasting and fasting plasma insulin and non-fasting plasma triglyceride and leptin levels compared to ND rats. No significant differences in plasma cholesterol parameters were noted either. Taken together, these data demonstrate that mild insulin resistance was induced by the high-fat diet.

Consistent with our previous rodent studies (Tahara et al., 2012a, 2012b, 2013), treatment with ipragliflozin increased urinary glucose excretion and urine volume (Figure 6A, B) and significantly decreased fasting plasma glucose and fasting plasma insulin levels (Figure 5A, B). No changes were noted in the non-fasting plasma glucose level, but non-fasting plasma insulin tended to be decreased \( (P=0.11) \). While non-fasting plasma triglyceride was improved with ipragliflozin treatment, neither plasma total cholesterol nor HDL-cholesterol was (Table 1). Levels of plasma leptin, which are associated with the amount of adipose tissue, were also reduced on ipragliflozin treatment (Table 1).

Plasma NEFA and 3-hydroxybutyrate were significantly higher in fasted ipragliflozin-treated animals (Figure 5C, D), as too was urinary 3-hydroxybutyrate excretion (Figure 6C). The increase in plasma levels of NEFA and 3-hydroxybutyrate suggests the enhancement of lipolysis and fatty acid oxidation, respectively. In the non-fasted state, the effects of ipragliflozin on plasma NEFA and 3-hydroxybutyrate levels were not clear, suggesting that feeding may influence these effects.

Treatment of ipragliflozin did not affect indicators of renal glomerular function such as plasma creatinine level, urinary creatinine excretion, or creatinine clearance (Table 1), nor was any change in hematocrit levels detected after the treatment of ipragliflozin (Supplementary Figure 2),
demonstrating that dehydration did not occur.

3.3. Effects of ipragliflozin on respiratory exchange ratio and heat production

To determine the effects of ipragliflozin on whole-body metabolism, indirect calorimetry in the dark phase was measured. Ipragliflozin did not influence O₂ consumption but did tend to decrease CO₂ production (\( P=0.055 \)) (Figure 7A). The respiratory exchange ratio was significantly lowered by ipragliflozin (Figure 7B). Although the total heat production rate (HPR) did not differ markedly between the ipragliflozin- and vehicle-treated groups, HPR from glucose did decrease while that from fat was markedly increased after ipragliflozin treatment (Figure 7C, D).

4. Discussion

Here, we investigated the effects of ipraglifozin on body weight and body composition as well as examined the mechanism responsible for body weight reduction in HFD rats. Chronic treatment of ipragliflozin for four weeks suppressed any increase in body weight despite a slight increase in food intake. Tissue weights and DEXA and CT findings demonstrated that the reduction in body weight was accompanied by reduced visceral and subcutaneous fat masses but not lean mass or bone mineral content. Analysis of plasma and urinary parameters further indicated that ipragliflozin increased urinary glucose excretion and improved hyperinsulinemia, hypertriglyceridemia, and hyperleptinemia. Ipragliflozin also increased plasma NEFA and 3-hydroxybutyrate and the urinary excretion of 3-hydroxybutyrate, particularly in the fasted state, suggesting possible enhancement of
lipolysis and fatty acid oxidation. Indirect calorimetry showed that ipragliflozin lowered the respiratory exchange ratio and decreased the HPR from glucose but increased it from fat, indicating that ipragliflozin mainly promoted the use of fatty acids instead of glucose as an energy source without changing the whole-body energy consumption. Taken together, these findings suggest that weight loss with ipragliflozin may primarily result from a reduction in fat tissue content via enhanced fatty acid utilization. Reductions in body weight with a concomitant increase of urinary glucose excretion have been previously shown in SGLT2-deficient mice (Jurczak et al., 2011), suggesting that the body weight reduction induced by ipragliflozin is due to the inhibition of SGLT2.

In the present study, ipragliflozin caused osmotic diuresis due to glucose excretion in the urine (Figure 6A), raising the possibility that the body weight reduction might be due to water loss. Although we did not directly measure whole-body fluid content, we did find that ipragliflozin does not alter hematocrit levels, an indicator of dehydration (Supplementary Figure 2), suggesting that the body fluid content was only negligibly affected by ipragliflozin. We also confirmed that ipragliflozin actually increased water consumption to compensate for water loss due to osmotic diuresis (data not shown). Thus, fluid depletion was not caused by ipragliflozin, thereby suggesting this route was not a major contributing factor to body weight reduction.

Although ipragliflozin and several SGLT2 inhibitors such as dapagliflozin reduce body and total fat weight in HFD animals (Tahara et al., 2013; Devenny et al., 2012; Liang et al., 2012), their effects on visceral and subcutaneous fat masses have not been assessed separately. Reducing visceral fat
mass is an important health outcome, given the growing body of literature emphasizing that visceral adiposity rather than subcutaneous adiposity is associated with increased risk of diabetes, insulin resistance, and cardiovascular disease (Despres, 2012; Unger, 1995; Trayhurn et al., 2011; da Silva, 2009). Therefore, in addition to measuring whole-body composition, we also analyzed the effects of ipragliflozin on visceral and subcutaneous fat masses in the abdominal area using small-animal CT (Luu et al., 2009; Hillebrand et al., 2010). Treatment with ipragliflozin significantly reduced the visceral fat mass and tended to decrease the subcutaneous fat mass compared to the vehicle group, and the percentage reductions among these masses were similar (Figure 4C, D). Recently, Bolinder et al. (2012) reported by magnetic resonance that chronic treatment of dapagliflozin in patients with type 2 diabetes mellitus produces mean reductions in visceral and subcutaneous fat masses. Thus, based on these clinical findings for dapagliflozin and results from our present animal study, reductions in body weight by ipragliflozin in clinical studies should also be accompanied by reduced visceral and subcutaneous fat masses.

Theoretically, a change in body weight arises from a shift in balance of intake, energy consumption, and overall loss of calories. Because total energy consumption did not change after ipragliflozin treatment in the indirect calorimetry (Figure 7C), the reduction in body weight by ipragliflozin in HFD rats could be attributed to a net negative balance of calorie intake and loss. The average daily calorie intake of ipragliflozin-treated rats was 94.9 kcal/day, which was slightly larger than that of vehicle-treated rats (91.0 kcal/day) (Figure 1B). The effect of ipragliflozin on calorie absorption may be negligible, because the dose of ipragliflozin used in this study inhibited neither intestinal
glucose (Tahara et al., 2012b) nor lipid absorption (unpublished data) in rodents. Urinary glucose loss of ipragliflozin-treated rats averaged 4575 mg/day (Figure 6B), which corresponds to an energy loss of 17.0 kcal/day based on the heat of combustion of glucose (3.719 kcal/g) (Livesey and Elia, 1988). Energy loss as urinary glucose of vehicle-treated rats was negligible. Therefore, the daily net energy balance (intake-loss) in vehicle- and ipragliflozin-treated rats was 91.0 and 77.9 kcal/day, respectively, with the net energy balance in ipragliflozin-treated rats coming out to 85.6% that of vehicle-treated rats. Assuming 6.16 kcal/g body fat for rodents (Gurr, 1980), this deficit of 13.1 kcal/day in the ipragliflozin-treated group could explain the fat weight loss of 2.13 g/day and a 63.9 g total body weight loss after 30-day treatment. Interestingly, this degree of weight loss is consistent with the differences in actual body weight between the vehicle and ipragliflozin groups (61.8 g) (Figure 1A), strongly suggesting that ipragliflozin reduced fat weight by promoting negative energy balance in HFD rats.

Treatment of ipragliflozin in HFD rats caused a slight increase (about 4%) in average daily food intake, an effect similar to that observed on treatment with canagliflozin for 3 weeks in obese Zucker fatty rats (Liang et al. 2012). However, Devenny et al. (2012) reported contrasting findings, with chronic treatment of dapagliflozin for 34 days in HFD rats dramatically elevating food intake (approximately 30%), leading that group to conclude that this increase compensated for the energy loss suffered due to urinary glucose excretion. The reason for the discrepancy among the SGLT2 inhibitors is not clear. Differences may relate to differing experimental conditions such as animal model, composition of diet, or body weight before drug treatment. Alternatively, each SGLT2
inhibitor may affect food intake mechanisms differently. Future studies are needed to clarify the matter.

Given the present results, the increase in whole body fatty acid oxidation might account for, at least in part, the reduction in body fat by ipragliflozin. Although the precise mechanisms of action remain to be elucidated, a direct effect of ipragliflozin in main metabolic tissues (fat, liver, and skeletal muscle) is unlikely, because the SGLT2 protein was not detected in those tissues in rats (Sabolic et al., 2012) and ipragliflozin did not affect glucose transport in 3T3-L1 adipocytes, HepG2 hepatocytes, or L6 myotubes (IC\textsubscript{50}>1,000 nM) (unpublished data). In contrast, based on present and previous findings, the decrease in plasma glucose and insulin could be considered a trigger mechanism involved in the body fat reduction via urinary glucose excretion. Tahara et al. (2013) reported a decrease in plasma glucose and a concomitant decrease in plasma insulin immediately after single administration of ipragliflozin in non-fasted rodents. This decrease in plasma insulin levels is mainly explained as an acute suppression of insulin secretion from pancreatic beta cells via either or both removal of the glucose-stimulated insulin secretion (Jensen et al., 2008) or compromising a hypothalamic low-glucose-sensing mechanism following glucose deprivation (Marty et al., 2007; Osundiji et al., 2012). As shown by diazoxide, an inhibitor of insulin secretion, suppression of insulin secretion can enhance lipolysis in adipose tissue, attenuate hepatic lipogenesis, and increase the fat oxidation rate (Eaton and Schade, 1980; Alemzadeh and Tushaus, 2005; Alemzadeh et al., 2008). These effects are consistent with our findings that ipragliflozin causes an increase in plasma NEFA levels, decrease in plasma triglyceride levels, and a whole-body
energy oxidation shift to fatty acids. As a result, chronic enhancement of lipolysis may lead to a reduction in fat storage in the adipose. To clarify the detailed mechanism of fat loss by ipragliflozin, additional studies concerning changes in the activity and expression of key molecules involved in lipolysis, lipogenesis, or fatty acid oxidation in main metabolic tissues are needed.

In conclusion, our findings here demonstrate that ipragliflozin improves HFD-induced obesity by shifting the energy utilization from glucose to fatty acid during steady caloric loss through glucosuria. These results suggest potential benefit with using ipragliflozin beyond improving hyperglycemia in the treatment of type 2 diabetic patients. Clinical trials with ipragliflozin are currently in progress, with the expectation that the trials will clarify the suitability of ipragliflozin for the treatment of obese type 2 diabetes patients.

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Conflict of interest

M. Yokono, T. Takasu, Y. Hayashizaki, K. Mitsuoka, R. Kihara, Y. Muramatsu, S. Miyoshi, A. Tahara, E. Kurosaki, Q. Li, M. Sasamata, and M. Shibasaki are employees of Astellas Pharma Inc. H. Tomiyama is employee of Kotobuki Pharmaceutical Co. Ltd. Ipragliflozin is in clinical
development by Astellas Pharma Inc. and Kotobuki Pharmaceutical Co. Ltd.

References


triglyceride concentration in the rat. Diabetologia 18, 301-306.

Eckel, R.H., Kahn, S.E., Ferrannini, E., Goldfine, A.B., Nathan, D.M., Schwartz, M.W., Smith, R.J.,
Smith, S.R., 2011. Obesity and type 2 diabetes: what can be unified and what needs to be
individualized? Diabetes Care 34, 1424-1430.


Type 2 Diabetic Patients With Inadequate Glycemic Control by Diet and Exercise A randomized,
double-blind, placebo-controlled, phase 3 trial. Diabetes Care 33, 2217-2224.

Iib, randomized, placebo-controlled study of the SGLT2 inhibitor empagliflozin in patients with

Active- and placebo-controlled dose-finding study to assess the efficacy, safety, and tolerability of
multiple doses of ipragliflozin in patients with type 2 diabetes mellitus. J Diabetes Complications
27, 268-273.


Hillebrand, J.J., Langhans, W., Geary, N., 2010. Validation of computed tomographic estimates of
intra-abdominal and subcutaneous adipose tissue in rats and mice. Obesity (Silver Spring) 18,
848-853.


Table 1

Effects of ipragliflozin on non-fasting plasma and urinary parameters in high-fat diet-induced obese rats

<table>
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<tr>
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<th>Normal diet Vehicle</th>
<th>High-fat diet Vehicle</th>
<th>High-fat diet Ipragliflozin 10 mg/kg</th>
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<tr>
<td>Plasma triglyceride (mg/dL)</td>
<td>114.4 ± 15.5</td>
<td>256.4 ± 35.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143.3 ± 18.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Plasma total cholesterol (mg/dL)</td>
<td>62.3 ± 4.0</td>
<td>80.3 ± 7.6</td>
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<tr>
<td>Plasma HDL-cholesterol (mg/dL)</td>
<td>27.3 ± 1.8</td>
<td>33.7 ± 3.2</td>
<td>38.2 ± 4.1</td>
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<tr>
<td>Plasma leptin (ng/mL)</td>
<td>4.18 ± 0.38</td>
<td>12.82 ± 1.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.87 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma creatinin (mg/dL)</td>
<td>0.659 ± 0.064</td>
<td>0.633 ± 0.053</td>
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<tr>
<td>Urinary creatinine excretion (mg/day)</td>
<td>15.5 ± 0.8</td>
<td>15.2 ± 0.4</td>
<td>14.5 ± 0.3</td>
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<tr>
<td>Creatinine clearance (mL/min)</td>
<td>1.76 ± 0.21</td>
<td>1.73 ± 0.12</td>
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Ipragliflozin was orally administered to high-fat diet-induced obese rats once daily for 4 weeks, and non-fasting plasma and urinary parameters were measured at Week 3. Values are expressed as mean ± S.E.M. of eight animals in each group. <sup>a</sup><i>P</i> < 0.05 vs. normal diet vehicle group. <sup>b</sup><i>P</i> < 0.05 vs. high-fat diet vehicle group.
Figure legends

**Figure 1.** Effects of ipragliflozin on (A) body weight and (B) food intake in high-fat diet-induced obese rats. Ipragliflozin was orally administered to obese rats for 4 weeks. Values are mean ± S.E.M. for 8 animals in each group. #P < 0.05 vs. normal diet group, *P < 0.05 vs. high-fat diet vehicle group.

**Figure 2.** Effects of ipragliflozin on (A) epididymal, (B) retroperitoneal, (C) mesenteric fat, (D) liver, and (E) kidney weights in high-fat diet-induced obese rats. Ipragliflozin was orally administered to obese rats for 4 weeks. Values are mean ± S.E.M. for 8 animals in each group. #P < 0.05 vs. normal diet group. *P < 0.05 vs. high-fat diet vehicle group.

**Figure 3.** Effects of ipragliflozin on body composition in high-fat diet-induced obese rats. Whole-body composition was measured with dual-energy X-ray absorptiometry (DEXA) analysis after a 4-week treatment of ipragliflozin. (A) whole-body weight, (B) body fat mass, (C) lean body mass, and (D) bone mineral content are shown. Values are mean ± S.E.M. for 9-10 animals in each group. #P < 0.05 vs. normal diet group. *P < 0.05 vs. high-fat diet vehicle group.

**Figure 4.** Effects of ipragliflozin on abdominal fat mass in high-fat diet-induced obese rats. Ipragliflozin was orally administered to obese rats for 4 weeks. Fat mass in the abdominal region between the proximal end of the 1st and the distal end of the 6th lumbar vertebra (L1-L6) and the
1st sacral vertebra (S1) was analyzed using small-animal computed tomography (CT). (A) Typical image of CT analysis. Subcutaneous fat mass is indicated in orange, visceral fat in yellow, and excluded regions in green. (B) Whole abdominal fat volume, (C) visceral fat volume, and (D) subcutaneous fat volume are shown. Values are mean ± S.E.M. for 10 animals in each group. *P < 0.05 vs. normal diet group. *P < 0.05 vs. high-fat diet vehicle group.

**Figure 5.** Effects of ipragliflozin on non-fasting and fasting plasma parameters in high-fat diet-induced obese rats. Blood was collected 14 h after final dosing. Plasma levels of (A) glucose, (B) insulin, (C) non-esterified fatty acid (NEFA), and (D) 3-hydroxybutyrate are shown. Values are mean ± S.E.M. for 8 animals in each group. *P < 0.05 vs. normal diet group. *P < 0.05 vs. high-fat diet vehicle group.

**Figure 6.** Effects of ipragliflozin on urinary parameters in high-fat diet-induced obese rats. Urine was collected for 24 h after final dosing. (A) Urine volume, and urinary (B) glucose and (C) 3-hydroxybutyrate are shown. Values are mean ± S.E.M. for 8 animals in each group. *P < 0.05 vs. normal diet group. *P < 0.05 vs. high-fat diet vehicle group.

**Figure 7.** Effects of ipragliflozin on (A) oxygen consumption (VO₂) and carbon dioxide production (VCO₂), (B) respiratory exchange ratio (RER), (C) heat production rate (HPR), and (D) HPR from glucose and fat during dark phase in high-fat diet-induced obese rats. Indirect calorimetry
measurements were done after a 3-week treatment of ipragliflozin. Values are mean ± S.E.M. for 5-10 animals in each group. *$P < 0.05$ vs. high-fat diet vehicle group.
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
Figure 7.