Estriol blunts postprandial blood glucose rise in male rats through regulating intestinal glucose transporters

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1Department of Pharmacology, Toxicology and Therapeutics, School of Medicine, University of Kansas Medical Center, Kansas City, Kansas; 2College of Korean Medicine, Gachon University, Seongnam, Korea; 3Natural Products Research Institute, Korea Institute of Science and Technology, Gangneung, Gangwon-do, Korea; and 4Department of Biology, South University of Science and Technology of China, Shenzhen, Guangdong, China

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Yamabe N, Kang KS, Lee W, Kim SN, Zhu BT. Estriol blunts postprandial blood glucose rise in male rats through regulating intestinal glucose transporters. Am J Physiol Endocrinol Metab 308: E370–E379, 2015. First published December 17, 2014; doi:10.1152/ajpendo.00209.2013.—Despite increased total food intake in healthy, late-stage pregnant women, their peak postprandial blood sugar levels are normally much lower than the levels seen in healthy nonpregnant women. In this study, we sought to determine whether estriol (E3), an endogenous estrogen predominantly produced during human pregnancy, contributes to the regulation of the postprandial blood glucose level in healthy normal rats. In vivo studies using rats showed that E3 blunted the speed and magnitude of the blood glucose rise following oral glucose administration, but it did not appear to affect the total amount of glucose absorbed. E3 also did not affect insulin secretion, but it significantly reduced the rate of intestinal glucose transport compared with vehicle-treated animals. Consistent with this finding, expression of the sodium-dependent glucose transporter 1 and 2 was significantly downregulated by E3 treatment in the brush-border membrane and basolateral membrane, respectively, of enterocytes. Most of the observed in vivo effects were noticeable stronger with E3 than with 17β-estradiol. Using differentiated human Caco-2 enterocyte monolayer culture as an in vitro model, we confirmed that E3 at physiologically relevant concentrations could directly inhibit glucose uptake via suppression of glucose transporter 2 expression, whereas 17β-estradiol did not have a similar effect. Collectively, these data showed that E3 can blunt the postprandial glycemic surge in rats through modulating the level of intestinal glucose transporters.

estriol; 17β-estradiol; postprandial blood glucose level; SGLT1; GLUT2

While the total amount of food intake is markedly increased in late-stage pregnant woman (20), their 2-h postprandial blood sugar levels rarely exceed 120 mg/dl during a healthy pregnancy, which are markedly lower than what are commonly seen in healthy nonpregnant women (15, 19, 35). Earlier studies have shown that the blunting of the postprandial blood glucose surge during pregnancy is highly beneficial for reducing the incidence of macrosomia (i.e., abnormal, excessive fetal growth), because surging maternal and fetal glucose levels often are accompanied by episodic fetal hyperinsulinemia, which promotes excess nutrient storage and ultimately macrosomia (18, 34). Consistent with this suggestion, a number of clinical studies (35, 38, 39) have shown that when the maternal 2-h postprandial blood glucose levels are maintained below 120 mg/dl, only ~20% of fetuses have macrosomia; but if the postprandial glucose levels are elevated (between 120 and 160 mg/dl), the incidence of macrosomia becomes significantly higher (35%). Similarly, if the maternal postprandial insulin response is inadequate, usually it would result in recurrent postprandial hyperglycemic episodes, which also contribute to abnormal, accelerated fetal growth.

The endogenous estrogens, such as 17β-estradiol (E2) and estrone, play an important role in regulating various physiological and pathological states in women as well as men (26). Studies have shown that the endogenous estrogens can modulate glucose homeostasis and insulin resistance (16, 24). While E2 and estrone are the quantitatively most important estrogens produced in a nonpregnant woman, E3 is the quantitatively most important estrogen produced during pregnancy, and its circulating concentrations at late stages of pregnancy can reach 1,000 times higher than those seen in a nonpregnant woman (3, 32). During pregnancy, virtually all of the E3 in the maternal circulation is synthesized in the feto-placental unit, and the measurement of the total E3 has been shown to be useful in monitoring the fetal well-being (4, 13). Interestingly, it was reported many years ago that combination of normoglycemia and normal E3 excretion during pregnancy is associated with a more favorable fetal outcome, whereas combination of abnormal glucose tolerance and abnormal E3 excretion is associated with significantly higher incidences of fetal growth abnormalities and perinatal death (1). The potential role of E3 in modulating postprandial blood glucose surge and insulin response, however, is not known at present and thus is the subject of our present investigation.

It is known that estrogen receptors (ERs) mediate many of the biological actions of estrogen in various target organs, including the gastrointestinal tract (2, 8, 40). However, it is presently unclear whether estrogens act through ERs to modulate intestinal glucose absorption carried out by the sodium-dependent glucose transporter 1 (SGLT1) in the brush-border membrane and the glucose transporter 2 (GLUT2) in the basolateral membrane (42). In this study, we have also probed the effect of E2 and E3 on intestinal glucose uptake and glucose transporter expression and the influence of an ER pure antagonist, ICI-182,780, on these changes. Our results showed that E3 can blunt the postprandial blood glucose surge in rats through modulating the level of intestinal glucose transporters.
EFFECT OF ESTRIOL ON POSTPRANDIAL GLUCOSE LEVELS

MATERIALS AND METHODS

Animal Experiments

All procedures involving the use of live animals as described in this study were approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center, and the National Institutes of Health guidelines for humane treatment of animals were followed. Male Sprague-Dawley rats weighing ~150 g, at 5–6 wk of age, were obtained from Harlan Laboratories (Indianapolis, IN). They were exposed to a 12:12-h light-dark cycle and had free access to a standard laboratory rat chow and water. After ~1 wk of acclimatization, the animals were weighed and then randomly divided into three groups: vehicle treatment group, E2 treatment group, and E3 treatment group. The dose of E2 and E3 (obtained from Steraloids, Newport, RI) used in this study was 500 μg/kg body wt (oral gavage once daily, dissolved in 1% methylcellulose), which was selected according to an earlier study (23) that assessed the cholesterol-lowering effect of estrogens. Rats were housed in metabolic cages equipped with system to measure total intake of feed and water. The body weight, food intake, and water intake were measured every day during the treatment period. After 15 days of E2 or E3 treatment, blood samples were collected in heparinized tubes by cardiac puncture of anesthetized rats. Plasma samples were prepared immediately by centrifugation. After cardiac puncture for blood collection, the rats were perfused with ice-cold saline, and the entire small intestine was collected for ex vivo bioassays, immunohistological evaluation, and other measurements (described later).

Measurement of Plasma Parameters

Plasma glucose, insulin, and total cholesterol levels were determined using the Autokit Glucose (Wako Diagnostics, Richmond, VA), Cholesterol E-Test (Wako Diagnostics), and the Ultra-sensitive Rat Insulin ELISA Kit (Crystal Chemical, Downers Grove, IL), respectively.

To determine the plasma levels of unbound (free) E2 and E3 in rats receiving E2 or E3 oral administration, a separate experiment was conducted. On day 15 of the continual estrogen treatment, the animals were anesthetized using isoflurane and blood samples were collected from the tail vein immediately before and 1 or 2 h after E2 or E3 oral administration. The free plasma levels of E2 and E3 in each sample were determined using ELISA kits (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s procedures. The detection sensitivity for E2 and E3 in these ELISA kits ranged from 14 to 19 pg/ml.

Glucose Tolerance Tests

On day 7 of estrogen treatment, the oral glucose tolerance test (OGTT) was performed for all rats in different treatment groups. Rats were fasted for 15 h before OGTT test. A fasting blood sample was first taken through direct puncture of the tail vein of each animal, and then six more blood samples were collected at the 1-, 3-, 5-, 15-, 30-, and 60-min intervals following oral administration of a glucose solution containing 10% (wt/vol) glucose (at a dose of 1 g/kg body wt) (41). Heparin-containing blood samples were immediately centrifuged, and plasma was separated and then frozen at ~20°C until analyzed for glucose and insulin levels.

On day 12 of estrogen treatment, the intravenous glucose tolerance test (IVGTT) was performed for all rats in different treatment groups. Rats were fasted for 15 h before the IVGTT test. A fasting blood sample was first taken through direct puncture of the tail vein of each animal, and then six more blood samples were collected at the 1-, 3-, 5-, 15-, 30-, and 60-min intervals following intravenous injection of 50% (wt/vol) glucose (at a dose of 0.5 g/kg body wt) (41). Heparin-containing blood samples were immediately centrifuged, and the plasma was separated and then frozen at ~20°C until analyzed for glucose and insulin levels.

The areas under curves (AUCs) for glucose and insulin were calculated according to the trapezoidal rule between 0 and 60 min during the OGTT and IVGTT (21).

Measurement of Intestinal Glucose Transport Ex Vivo

At the end of the estrogen treatment on day 15, the entire small intestine was quickly isolated and transferred to normal saline at 5–10°C. The proximal and distal segments were identified from 20 cm distal to the pyloric sphincter (proximal) and a second segment that extended from 20 cm proximal to the ileocolonic junction (distal) and then cut into 10-cm lengths and kept in cold normal saline solution (17). The everted sac technique was used in the ex vivo experiment as described previously (27). Briefly, each sac (4 sacs for each condition) was made by tying one end of the segment, filling it with 1 ml/10 cm (of the segment) of Krebs bicarbonate solution (containing 120 mM NaCl, 4.5 mM KCl, 1 mM MgSO4, 1.8 mM NaH2PO4, 0.2 mM NaHPO4, 1.25 mM CaCl2, 25 mM NaHCO3, and 5.5 mM glucose) and then tying the other end. The sacs were immersed in 40 ml of Krebs solution (containing 120 mM NaCl, 4.5 mM KCl, 1 mM MgSO4, 1.8 mM NaH2PO4, 0.2 mM NaHPO4, 1.25 mM CaCl2, 25 mM NaHCO3, and 30 mM glucose) in a flask and incubated in a shaker bath (30 min, 37°C/40 rpm) (43). The sacs were then removed, blotted, weighed, and opened, and the changes of glucose concentration in their contents (serosal fluid) and the incubating media (mucosal fluid) were analyzed as described above. Throughout the entire procedure, the mucosal solution was bubbled with O2/CO2 (95/5) gas.

Immunohistochemical and Real-Time PCR Analyses

The small intestine collected from the treated rats was fixed overnight in 10% phosphate-buffered formalin at 4°C,embedded in paraffin, and then cut into 5-μm sections. The slides from all experimental groups were deparaffinized with three changes of xylene and rehydrated through a graded ethanol series to distilled water. Antigen retrieval was performed by placing the slides in 10 mM citrate buffer (pH 3.0), heating in a microwave oven for 20 min, and allowing the slides to cool to room temperature for 20 min. Slides were rinsed once with PBS, and endogenous peroxidase activity was blocked by incubating the samples for 30 min with 3% H2O2 in PBS, followed by rinsing three times with PBS. Nonspecific binding was blocked by incubating the slides for 30 min in 2% normal goat serum (Vector Laboratories, Burlingame, CA) in 1% Triton X-100 containing PBS, followed by incubation with specific antibodies (Millipore, Billerica, MA; 1:200 dilution) against SGLT1 or GLUT2 in the blocking solution as described above. Then, slides were incubated with FITC-conjugated anti-rabbit IgG (Vector Laboratories; 1:100 dilution for GLUT2 and 1:300 dilution for SGLT1) in the blocking solution as described above and mounted in Vectashield hard set mounting media with DAPI (Vector Laboratories). Negative controls without the primary antibody were also done for each experiment. To perform quantitative evaluation of immunostaining, three to four sections per animal were selected, and the images were captured and analyzed using the Axiostar image analysis software. One field (100 × 100 μm) in each slide within the brush-border and enterocyte basolateral membrane regions was selected for quantification, and the intensity of immunoreactivity was evaluated according to the relative optical density value. Immunohistochemical slides were reviewed in a blinded manner.

To determine the levels of rat rSGLT1 and rSGLT2 mRNAs in the rat intestine, real-time quantitative PCR analysis was used. First, the total RNAs were isolated from collected tissues using the QIAgen RNeasy Kit (Qiagen, Valencia, CA) by following the manufacturer’s instructions. The RNA concentration of each sample was determined by spectrophotometric analysis (at 260 nm). The integrity of each RNA sample was evaluated using the Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA). cDNA synthesis was performed with 1 μg of total RNA in 20 μl using random primers.
In Vitro Glucose Uptake Assay Using Caco-2 Cell Monolayers

Caco-2 cells were obtained from American Type Cell Collection (ATCC, Manassas, VA) and cultured in DMEM (HyClone Laboratories, Logan, UT) containing 10% FBS (HyClone Laboratories), 1% antibiotics (HyClone Laboratories), 1× nonessential amino acids (WelGENE, Daegu, Korea), and 1 mM sodium pyruvate (WelGENE) at 37°C with 5% CO2 in air.

For differentiation of Caco-2 cells into monolayers in culture, cells were seeded in 96-well black plates (Corning, NY) and cultured for 6–7 days. Then, the cells were incubated for an additional 24 h in the absence or presence of E2 or E3. Subsequently, cells were incubated with 50 μM 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (2-NBDG; Invitrogen) for 15 min and then washed with PBS three times to remove free 2-NBDG. The fluorescence intensity of cells containing 2-NBDG was measured on the Infinite M1000 microplate reader (TECAN, Männedorf, Switzerland) with excitation at 485 nm and emission at 535 nm. To probe the involvement of ERs, cells were also cotreated with the anti-estrogen ICI-182,780 at 10-, 100-, and 1,000-nM concentrations.

To determine the expression of the genes listed in Table 1, total RNAs were isolated from cultured Caco-2 cells using Trizol reagent (Invitrogen). Real-time quantitative PCR analyses of these genes were performed as described above. The data were normalized according to the corresponding expression levels of 18s-actin. The quantitative data are expressed as means ± SD. The effect of E2 or E3 on each parameter was examined using the one-way ANOVA. Individual differences among groups were analyzed using the Dunnett’s test (SPSS 11.5 software). P < 0.05 was considered statistically significant.

Statistical Analysis

The quantitative data are expressed as means ± SD. The effect of E2 or E3 on each parameter was examined using the one-way ANOVA. Individual differences among groups were analyzed using the Dunnett’s test (SPSS 11.5 software). P < 0.05 was considered statistically significant.

RESULTS

Effects of E3 and E2 on Food Intake, Body Weight, and Levels of Blood Glucose and Insulin

The body weight of vehicle-treated control animals increased continually during the 15-day experimental period (Fig. 1A). Animals treated with 0.5 mg/kg E2 had a slightly reduced body weight gain compared with the control group, but the body weight gain of animals treated with 0.5 mg/kg E3 was significantly reduced throughout the experimental period (Fig. 1A). During the OGTT and IVGTT, there were noticeable decreases in the animal body weights in all treatment groups, which was largely due to the overnight fasting prior to each of the tests. Figure 1B shows the average amount of daily food intake of the animals during the 15-day experimental period. The average amount of food intake of vehicle-treated animals was 23.1 ± 2.8 g/day, and treatment of the animals with E2 or E3 markedly suppressed their food intake. The anorexic effect of E3 was stronger than that of E2 (Fig. 1B).

The basal plasma glucose and insulin levels in animals treated with E3 or E2 were not significantly different from vehicle-treated animals (Table 2). The total levels of plasma cholesterol were markedly reduced by treatment with E3 or E2, and E3 had a far stronger effect than E2 (Table 2).

In the morning of the day 15 of the treatment period before E2 or E3 administration, the plasma level of free E2 was found to be at 37.8 pg/ml (0.14 nM) and 30.0 pg/ml, respectively (Fig. 1C). At 1 and 2 h after E2 treatment, its level was increased to 60.1 pg/ml (0.22 nM) and 64.1 pg/ml (0.24 nM), respectively, and at 1 and 2 h after E3 treatment, its level was increased to 52.7 pg/ml (0.19 nM) and 45.7 pg/ml (0.17 nM), respectively (Fig. 1C). In comparison, at 1 and 2 h after E3 administration, the plasma level of free E3 was increased from 11.8 pg/ml (0.04 nM) to 619.7 pg/ml (2.15 nM) and 628.7 pg/ml (2.18 nM), respectively, although the plasma level of free E2 after E2 administration remained very low (Fig. 1D).

Effects of E3 and E2 on Glucose Tolerance

Oral glucose tolerance test. After 7 days of E3 or E2 treatment, the changes in plasma glucose and insulin levels were determined under both basal conditions and after oral glucose administration. As shown in Fig. 2, the fasting plasma glucose level of vehicle-treated rats was 64.5 ± 4.1 mg/dl, and it was significantly increased after 7 days of treatment with E2 or E3 (Fig. 2A). E3 had a similar effect as E2 (Fig. 2A). Next, to assess the modulating effect of estrogen treatment on postprandial blood glucose rise, we conducted the OGTT. As shown in Fig. 2B, when the vehicle-treated control animals were administered orally a water solution containing 10% (wt/vol) glucose, the plasma glucose level was increased rapidly to 175.2 ± 8.4 mg/dl in 30 min and then decreased to below 100 mg/dl at 180 min. However, this rapid increase in plasma glucose level was slightly attenuated in E2-treated rats but significantly attenuated in E3-treated rats; moreover, the plasma glucose levels of E3 or E2-treated rats remained at slightly higher levels (>100 mg/dl) before 180 min after oral glucose administration (Fig. 2B). It is evident that E3 is more

Table 1. Primers used in this study

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Oligonucleotide Sequence (5' → 3')</th>
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<tbody>
<tr>
<td>hGLUT2</td>
<td>GCTCATGCGCAATGACTG</td>
</tr>
<tr>
<td>Forward</td>
<td>AGCCATTGGACTGCGCCG</td>
</tr>
<tr>
<td>Reverse</td>
<td>AACCATTGGACTGCGGC</td>
</tr>
<tr>
<td>hGLUT1</td>
<td>TCCTGCGGCTTGAAGAT</td>
</tr>
<tr>
<td>Forward</td>
<td>AGAGAACCTGCCTCC</td>
</tr>
<tr>
<td>Reverse</td>
<td>GCTCATGCGCAATGACTG</td>
</tr>
<tr>
<td>gGAPDH</td>
<td>TCCTGCGGCTTGAAGAT</td>
</tr>
<tr>
<td>Forward</td>
<td>AGGTCCGGTTGAGGC</td>
</tr>
<tr>
<td>Reverse</td>
<td>AGAGAACCTGCCTCC</td>
</tr>
<tr>
<td>rGLUT2</td>
<td>AGCTTCCCGCGTTGAG</td>
</tr>
<tr>
<td>Forward</td>
<td>TAGCCAGAAGCCGCGACTCC</td>
</tr>
<tr>
<td>Reverse</td>
<td>TAGCCAGAAGCCGCGACTCC</td>
</tr>
<tr>
<td>rGLUT1</td>
<td>AGATGGATATGCCTCCTG</td>
</tr>
<tr>
<td>Forward</td>
<td>AGCTTCCCGCGTTGAG</td>
</tr>
<tr>
<td>Reverse</td>
<td>AGCTTCCCGCGTTGAG</td>
</tr>
<tr>
<td>β-Actin</td>
<td>AGGCTCTATGAGGGGC</td>
</tr>
</tbody>
</table>

GLUT2, glucose transporter 2; SGLT1, sodium-dependent glucose transporter 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; h, human; r, rat.
efficacious than E2 in reducing the magnitude of postprandial plasma glucose oscillation in these rats (Fig. 2B). Notably, the plasma glucose AUCs with or without E2 or E3 treatment were not significantly different (Fig. 2C), suggesting that the total amount of glucose absorbed likely was not different under different treatments.

Figure 2D shows the fasting insulin levels in the animals, and there were no significant differences among the different treatment groups. When the animals were orally administered the 10% (wt/vol) glucose, the blood insulin level in the vehicle-treated rats was increased rapidly to 1.6 ± 0.2 ng/ml (1,100%) in 30 min. However, this rapid increase in the plasma insulin level was significantly blunted in E3 or E2-treated rats (Fig. 2E). The attenuating effect of E3 against the rapid increase in plasma insulin level was slightly stronger than E2 (Fig. 2E).

According to the insulin AUCs, E3 strongly reduced the total amount of insulin released, and its effect was stronger than E2 (Fig. 2F).

**Intravenous glucose tolerance test.** Next, we sought to assess whether the blunting effect of estrogens on postprandial blood glucose level was related to their direct action in pancreatic β-cells. We determined the changes in plasma glucose and insulin levels in estrogen-treated animals during IVGTT. The fasting plasma glucose levels of vehicle-treated rats were not significantly different from rats treated with E2 or E3 (Fig. 3A), whereas fasting plasma insulin levels were significantly reduced by E2 or E3 administration (Fig. 3D). During IVGTT, administration of E3 or E2 did not affect glucose AUC value, and they also did not affect the plasma levels of insulin. These data suggest that estrogen treatment did not affect insulin release following intravenous injection of glucose (Fig. 3, B–F).

**Effects of E3 and E2 on Intestinal Glucose Transport**

Based on the results of OGTT and IVGTT, we hypothesized that treatment of animals with an estrogen may alter the rate of glucose absorption in the intestine, thereby blunting the rise of plasma glucose level following oral glucose administration. To test this hypothesis, we determined the effect of the in vivo estrogen treatment on the intestinal glucose transport. Treatment with E3 or E2 did not appreciably affect the intestinal weight and length (Fig. 4, A and B). In vehicle-treated rats, the
rate of glucose transport by the proximal intestine ex vivo was not significantly different from the distal intestine (Fig. 4, C and D). After in vivo treatment with E3 or E2 for 15 days, the serosal and mucosal transport of glucose ex vivo was significantly decreased compared with vehicle-treated group. It was apparent that E3 had a stronger effect than did E2 (Fig. 4, C and D).

Next, we determined the effect of in vivo estrogen treatment on intestinal glucose transporter levels. As shown in Fig. 5A, SGLT1 was highly expressed in the brush-border membrane of vehicle-treated rats, but its expression was downregulated after treatment with E3 or E2 for 15 days, and it was apparent that treatment with E3 produced a stronger downregulation than did E2 (Fig. 4, A and D). In addition, we found that GLUT2 was mainly localized in the basolateral membranes of enterocytes in vehicle-treated rats (Fig. 5B). Its expression was downregulated after treatment with E3 or E2. Similarly, a stronger downregulation of GLUT2 was observed in E3-treated animals compared with E2-treated animals (Fig. 5D). The decreases in intestinal SGLT1 and GLUT2 expression were also confirmed when they were measured using the real-time quantitative PCR analysis (Fig. 5, E and F).

Effects of E3 and E2 on Glucose Uptake and Glucose Transporter Expression in Caco-2 Cell Monolayers

To test the hypothesis that E3 and E2 directly act on intestinal epithelial cells and then alter the expression of the glucose transporters in these cells, we employed an in vitro model by using the differentiated human intestinal Caco-2 cell monolayer culture to compare the direct effect of E3 and E2 on glucose transport and glucose transporter expression. Caco-2 cells have been widely used to study glucose transport, and these cells were reported to express some of the glucose transporter mRNAs (14, 25). As shown in Fig. 6A, treatment of cultured Caco-2 cell monolayers with E3 produced a marked inhibition of glucose uptake, but this effect was not observed with E2. ICI-182,780 is a potent steroidal anti-estrogen, which can bind to both ERα and ERβ subtypes (11). Cotreatment of cells with ICI-182,780 abrogated E3-induced reduction in glucose uptake in the concentration-dependent manner (Fig. 6B).

Next, we also examined the effect of E2 and E3 on the expression of the human glucose transporters hGLUT2 and hSGLT1 in Caco-2 cells. We found that hGLUT2 expression

Fig. 2. Effect of E3 and E2 on OGTT. After overnight fasting, blood samples were collected from tail vein and plasma glucose (A) and insulin levels (D) were measured. Then, 10% glucose solution (1 g/kg body wt) was orally administered and blood samples were collected after 10, 30, 60, 90, 120, and 180 min and plasma glucose (B) and insulin levels (E) were measured. Incremental glucose area under the curve (AUC) value (C) and incremental insulin AUC value (F) were calculated. Values are means ± SD (n = 3 per group). Statistical significance was calculated by one-way ANOVA and multiple comparisons with Tukey’s HSD test. *P < 0.05 vs. vehicle-treated rats.
was readily detected in untreated Caco-2 cells, and its expression was reduced to a comparable extent as the inhibition of glucose uptake following treatment with 100 nM E3 (Fig. 6C). Similarly, the E3-induced inhibition of hSGLT1 expression in Caco-2 cells was restored by treatment with ICI-182,780 (Fig. 6C). Similar effects were not observed with E2 (Fig. 6C).

Notably, the cultured Caco-2 cells had basically an undetectable level of hSGLT1 expression under the experimental conditions used in this study (data not shown), and thus we did not further examine the modulating effect of E3 and E2 on its expression in these cells.

DISCUSSION

The clinical OGTT, which involves oral administration of 75 g glucose to a human subject, has been commonly used as a test for postmeal glucose response (31). In this study, we performed a similar OGTT in healthy male rats treated with an estrogen (E3 or E2) to examine whether estrogen can modulate postprandial glycemic oscillation. The rapid increase in blood glucose and insulin levels seen in vehicle-treated control rats was reduced by treatment with E3 or E2. To probe the mechanism by which E3 and E2 blunt postprandial glycemic oscillation, we conducted experiments to determine whether their effect is attributable to the regulation of the release of endogenous insulin. We found that E3 and E2 did not significantly affect the peak levels of blood glucose and insulin as well as their AUCs in rats following an intravenous injection of glucose. Based on these observations, we suspected that E3 and E2 might mainly affect the rate of intestinal glucose absorption. To test this hypothesis, we determined the effect of E3 or E2 treatment on the rate of intestinal glucose transport. There are two stages in intestinal glucose absorption occurring across enterocytes in vivo: the first is the uphill accumulation of glucose across the brush-border membrane by SGLT1, and the second is the downhill transport from the cell into blood through GLUT2 present in the basolateral membrane (42). Indeed, we found that after E3 administration, the relative rates of serosal and mucosal glucose transport were significantly reduced compared with vehicle-treated group. In further support of this hypothesis, we found that the level of SGLT1 and
GLUT2 expression in the enterocyte basolateral membrane and brush-border membrane, respectively, was downregulated after E3 and E2 treatment. The degree of their downregulation following treatment with E3 or E2 was consistent with the reduction in the rate of glucose transport. Collectively, these data suggest that the blunting effect of E3 and E2 on postprandial glycemic oscillations in normal rats is partly due to a reduced rate of gastric emptying, which needs further investigation. Nevertheless, the change in gastric emptying likely is not a major factor because our ex vivo study using the everted sacs showed that rats treated in vivo with estrogen have a significantly reduced rate of gastrointestinal glucose transport when tested ex vivo.

It was unclear whether estrogen acts directly on intestinal enterocytes to exert the effect or it acts indirectly, i.e., the estrogen may act on other organs or cells first to induce the release of an effector, which then acts on the enterocytes. To probe these possibilities, we used the differentiated human intestinal Caco-2 cell monolayer cultures as an in vitro model to compare the direct effect of E3 and E2 on glucose transport and glucose transporter expression. The differentiated Caco-2 cell monolayer cultures have been often used to study glucose transport (14, 25). When these cells are differentiated under certain conditions, their phenotype, both morphologically and functionally, closely resembles the small intestinal enterocytes (12, 28, 33). In this study, we found that treatment of differentiated Caco-2 cell monolayer cultures with E3 caused a significant inhibition of glucose uptake, and this effect nearly paralleled its effect on the expression of GLUT2 in these cells. These similarities suggest that the inhibition of intestinal enterocyte glucose absorption in vivo by E2 likely is mediated by its direct suppression of the expression of glucose transporters. Surprisingly, while E2 is moderately effective in vivo, it does not have an appreciable effect on glucose transport and GLUT2 expression in vitro. The observed in vivo effect of E2 likely is due to its metabolic conversion to E3, which is known to occur in rats (22, 36, 37).

Notably, E3, which is a much weaker estrogen than E2 (46), has a strong effect in reducing the expression of intestinal glucose transporters and glucose uptake in vivo and in vitro, whereas E2 only has a weaker effect in vivo and no effect in vitro. The mechanism for the differences in the opposite efficacy of E3 and E2 is not understood at present. It has been reported that ERβ is the predominant ER subtype expressed in human colon enterocytes and Caco-2 cells, although very low levels of ERα are also detected in these cells (5). Our earlier study showed that E3 has a preferential binding affinity for ERβ over ERα (by a factor of 3 to 1), whereas E2 has nearly the same binding affinity for both ERα and ERβ (46). It is possible that the preference of E3 for binding and activation of the ERβ system (the predominant ER subtype in enterocytes) may partly contribute to the stronger effect of E3 in enterocytes. This possibility is somewhat in line with the observed circulating levels of E2 and E3. After administration of 500 µg E2, the blood level of free E2 (which is the active form of estrogen) is only slightly increased, likely due to its extensive binding to the circulating proteins (e.g., sex hormone-binding globulin and albumin) (29). In comparison, after administration of the same amount of the less-hydrophobic E3, the blood level of free E3 is markedly increased, which would favor its strong preferential activation of the ERβ system in enterocytes.

Another possible explanation for the observed differential effect of E3 and E2 may relate to the involvement of additional signal pathway(s) in enterocytes other than ERα and ERβ. In recent years, there is growing support for the concept that certain biologically active estrogen metabolites formed in the body under certain physiological or pathophysiological conditions (such as during pregnancy) may serve as new hormones or as local chemical mediators that exert a distinct profile of biological functions (discussed in Ref. 45). E3 has been sug-
gested to be one of such bioactive estrogen metabolites in light of the fact that this endogenous estrogen derivative has many unique biological functions related to pregnancy that are not similarly shared by E2 (9, 10, 44).

**Conclusion**

In the present study, we investigated the role of E3 in modulating postprandial blood glucose level and insulin response in a rat model. We found that E3 blunts postprandial glycemic rise by slowing down the rate of intestinal glucose transport. This effect is achieved through downregulation of the rat intestinal SGLT1 and GLUT2 expression. The observation provides new insights into the mechanism underlying the beneficial effects of estrogens on postprandial blood glucose rise.

**GRANTS**

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**Fig. 5.** Effect of E3 and E2 on intestinal glucose transporter levels. At the end of the animal experiment after the animal were euthanized, whole intestines were collected from each animal, and immunofluorescence staining for sodium-dependent glucose transporter 1 (SGLT1; A) and glucose transporter 2 (GLUT2; B) were performed and quantified for rat (r)SGLT1 (C) and GLUT2 (D), respectively, as described in MATERIALS AND METHODS. The expression of the SGLT1 (E) and GLUT2 (F) genes were also analyzed using the real-time quantitative PCR. The expression level was analyzed by real-time quantitative PCR and normalized to the expression of actin. Values are means ± SD (n = 3 per group). Statistical significance was calculated by one-way ANOVA and multiple comparisons with Tukey’s HSD test. *P < 0.05 vs. vehicle-treated rats. #P < 0.05 vs. E2-treated rats.

**Fig. 6.** Effect of E2 and E3 on glucose transport and cell growth in human intestinal Caco-2 cells. A: glucose uptake in Caco-2 cells treated with E2 or E3 was measured with 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (2-NBDG). Each bar represents the means ± SD of triplicate experiments. B: glucose uptake in Caco-2 cells treated with an estrogen (E2 or E3) or in combination with an anti-estrogen (ICI-182,780) was measured using 2-NBDG. Each bar represents the means ± SD. C: expression of human glucose transporter 2 gene (hGLUT2) in Caco-2 cells was analyzed by the real-time quantitative PCR (n = 3) and normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Each bar represents the means ± SD. *P < 0.05 vs. vehicle-treated cells. #P < 0.05 vs. E2-treated rats.
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