Estrogen regulates duodenal glucose absorption through the effect of estrogen receptor-α on glucose transporters

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Abstract

Estrogen deficiency is an important reason for the obesity of menopause, but the specific mechanism is unclear. Here, we investigated the effect of estrogen on glucose absorption. The experiments were performed in human, mice and SCBN cells. We first observed the correlation between estrogen and blood glucose in women, which found that blood glucose was significantly higher in the premenstrual phase than in the preovulatory phase of young women. Similarly, with serum estradiol level decreased in ovariectomized (OVX) mice, ER-α and ER-β in the duodenum reduced, the weight, abdominal fat and blood glucose increased significantly. However, interestingly, the expression of SGLT1 and GLUT2 and the glucose absorption in duodenal decreased significantly. It was further confirmed that estrogen could significantly up-regulate the expression of SGLT1 and GLUT2 in SCBN cells, and this trend can be reversed after silencing ER-α, but it doesn't work after silencing ER-β, suggesting ER-α may be the key receptor of estrogen regulating glucose transporter. Mechanism study found that estrogen downstream can activate PKC pathway. Overall, our findings indicate that estrogen promotes glucose absorption, estrogen and its receptor (ER-α) deficiency can inhibit the expression of SGLT1 and GLUT2 through PKC signaling pathway, thereby reducing the glucose absorption.

Introduction

Nowadays, obesity has become the most important global public health problem. Worldwide obesity has nearly tripled since 1975. The prevalence of obesity was generally higher for women than for men in adults (World Health Organization). The risk in prevalence of obesity was significantly increased after menopause in women and some related studies have shown that midlife women commonly experience an increase in obesity after menopause and the lack of estrogen is an important factor in triggering obesity in menopausal women[1, 2]

Estrogen is a steroid hormone produced by both men and women and has a wide range of physiological functions. It is currently believed that the activation of estrogen can inhibit obesity. On the one hand, it can regulate peripheral energy and glucose homeostasis, prevent liver steatosis, improve insulin sensitivity, enhance skeletal muscle lipid oxidation, and regulate the distribution of adipose tissue; On the other hand, it also inhibits food intake and increases energy expenditure in the central nervous system, thereby preventing obesity caused by high-fat diet[3, 4]. However, estrogen deficiency will not only lead to impaired energy, glucose, and lipid metabolism in postmenopausal women[5, 6], but also increase food intake and reduce energy expenditure through interaction with orexin and anorexic hormones[7].

Although our previous studies have shown that estrogen receptor (ER) is expressed in mucosal epithelial cells along the digestive tract[8], there are currently few studies on the absorption of glucose by estrogen, and the specific mechanism is still unclear. The absorption of glucose is mainly in the small intestine, which is mainly through two pathways, one is sodium dependent active transport mediated by sodium dependent glucose transporter(Sodium-Glucose Cotransporter 1, SGLT1), the other is facilitated diffusion, which is mainly mediated by glucose transporter 2 (GLUT2)[9]. However, there is still no direct evidence to
show whether estrogen is involved in regulating the absorption of glucose by the glucose transporter of small intestinal mucosal epithelial cells.

Therefore, this study focuses on the role of estrogen in regulating duodenal glucose absorption and its molecular mechanism, which provides us with a new theoretical basis for further research and prevention of obesity, and also provides a basis for the clinical application of estrogen drugs.

Materials And Methods

1.1 Human blood glucose experiment

In this study, healthy Chinese women aged 20-30 years with regular menstrual cycle (menstrual period within 3-7 days, menstrual cycle in the range of 25-32 days) and without pelvic organic lesions or medical diseases causing irregular menstruation were selected. The research in healthy human volunteers was carried out according to the principles stipulated in the Declaration of Helsinki. The experimental protocol has been approved by the Human Subjects Committee in Affiliated Hospital of Zunyi Medical University, China. The blood glucose was measured during the menstrual period (the lowest level of estrogen in the body) and the ovulation period (the highest level of estrogen in the body). Before the measurement, fasting for 12 hours was required, and after fasting and oral glucose 75g, the participants’ peripheral blood glucose should be monitored at 5 min, 15 min, 30 min, 45 min, 60 min, 75 min and 90 min respectively to judge the change of blood glucose level.

1.2 Establishment of ovariectomy animal model

C57BL/6 female mice with 6 weeks of age (~17±2.2 g) were purchased from Beijing Huafukang Biotechnology Company and housed in the experimental animal facility of Zunyi Medical University under standard care conditions. Sexual maturity female mice (n=60) were randomly divided into ovariectomized (OVX) mice and sham-operated (Sham)mice, and were subjected to ovariectomy and sham operation after anaesthetization by intraperitoneal injection of 50 mg/kg ketamine and 10 mg/kg xylazine. Murine ovariectomy was performed in the OVX mice according to a previous study[10]. From the second day after the operation, the daily weight changes of the two groups were observed and recorded until 2 weeks after the operation. All animal experiments in this study have been approved by Committees on Investigations Involving Animals in Zunyi Medical University, China (KLLY(A)-2019-105), in strict accordance with Guidelines of the Committee on the Care and Use of Laboratory Animals.

1.3 Determination of serum estradiol

Two weeks after the operation, 10 mice were randomly selected from the two groups to collect their vena cava blood, and used it to determine their serum estradiol levels by radioimmunoassay. According to the instructions of the radioimmunoassay kit (Beijing Northern Institute of Biotechnology),
radioimmunoassay counter was used to measure the radiation intensity and calculate the estradiol content.

1.4 Mouse oral glucose tolerance test (OGTT)

Two weeks after the operation, 20 mice were randomly selected from the two groups. After fasting for 12 hours, glucose (3g/kg) was given by gavage. Blood was collected from the tail vein at 0, 5, 15, 30, 45, 60, 75, and 90 minutes, and blood glucose changes in mice were detected by a blood glucose meter (Roche Biotechnology). After the experiment, the mice were euthanized and the duodenum of the mice was collected.

1.5 Ussing chamber experiments

Ussing chamber experiments were performed as previously described to measure glucose absorption ex vivo in mice duodenum[11]. Specific operations are as follows: The distal duodenum of the mice, about 1.5 cm in length, was placed in isotonic mannitol solution at 4 °C. The intestinal segment was placed on a flat paraffin block with a sealing membrane on the surface, and the intestinal tube was submerged with the above-mentioned isotonic mannitol solution, then it was cut along the mesentery, and the serous and muscular tissues were stripped off. The mucosa and submucosa were fixed on the chamber (effective penetration area was 0.16 cm²), and then assembled with chamber. The mucosa side (top side) was added with 10 ml of working fluid, and the continuous and uniform 100% oxygen was perfused. 10 ml of working solution was added to the serosal side (basal side), and the mixture of 95% oxygen and 5% carbon dioxide was infused continuously and evenly. After assembly, the short-circuit current (Isc) was recorded every 5 minutes. After stabilizing for 15 minutes, glucose solution (20mM) was added to the mucosal side, and the Isc was continuously observed and record for 30 min. Then the changes of Isc was compared. The difference of Isc could reflect the index of tissue absorption (the difference between the highest value and the basic value).

1.6 Immunochemistry

The duodenum tissues of the two groups of mice were taken, fixed with formalin and embedded with paraffin. Immunohistochemical staining for ER-α, ER-β and P75 in the paraffin-embedded tissue blocks was done according to the previous method[12]. Anti-ER-α (1:50; Abcam), Anti-ER-β (1:50; Abcam), Anti-SGLT1 (1:100; Abcam), Anti-GLUT2 (1:100; Abcam) was used to detect the distribution and location of ER-α, ER-β, SGLT1, GLUT2 in duodenal mucosal epithelial tissue. Anti-P75 (1:50; Abcam) was used to detect the distribution and location of P75 in pancreatic tissue.

1.7 Cell experiments
SCBN cells, a non-tumorigenic duodenal epithelial cell line obtained from a human patient, which was a gift from Dr. Hui Dong in University of California San Diego. All cells were grown in DMEM medium supplemented with 10% fetal calf serum in an incubator with 5% CO₂ at 37°C.

1.8 shRNA

To silence ER or ERβ, lentivirus-based short hairpin RNA (shRNA) was used. SMARTvector lentiviral human ER-α shRNA (ctCTACTT CATCGCATTCCTT), human ER-β shRNA (CGGCAGACCACAAGCCCAAAT), and SMARTvector non-targeting control particles (TTCTCCGAACGTGTCACGT) were purchased from Shanghai Genechem Co.,Ltd. SCBN cells were transfected according to the manufacturer's protocol and western blot was used to detect the protein expression of ER-α and ER-β to demonstrate the success of silencing.

1.9 Western blotting

Murine duodenal mucosal tissues were taken at the time described previously. SCBN cells or ER-α or ER-β silencing SCBN cells were treated with 17β-estradiol (10 nM), PKC agonist PMA (200 μM) or PKC inhibitor Gö6976 (200 μM). Duodenal mucosal tissue or SCBN cells were homogenized respectively in lysis buffer at 4°C. The Western blot procedure for protein expression was performed as described above[12]. Anti-ER-α (1:100; Abcam), Anti-ER-β (1:100; Abcam), anti-SGLT1 (1:500; Abcam), anti-GLUT2 (1:1000; Abcam), Anti-P-PKC (1:500; Abcam), anti-P75 (1:2500; Abcam) or anti-GAPDH (1:5000; Abcam) was used as primary antibodies, and the results was expressed as the ratio relative to GAPDH.

1.10 Statistical analysis

The experimental data were collected and organized according to the requirements of a completely randomized control design, and all data were expressed as mean ± standard deviation. The data were analyzed by unpaired t-test with Graphpad prism 7.0, and P<0.05 indicated that the difference was statistically significant.

Results

2.1 Effect of estrogen on human blood glucose

As we all know, normal female estrogen changes periodically in different stages of the menstrual cycle. Typically, the first seven days of the menstrual cycle (early follicular phase) are characterized by low serum levels of estradiol. With the rise of a dominant follicle, estradiol levels rapidly increase during the second week of the menstrual cycle (late follicular phase). The estradiol peak is followed 12–24 h later by the luteinizing hormone surge, and ovulation[13]. In our previous study, we have found that estrogen level is lower in the premenstrual phase than in the preovulatory phase[14]. Herein we tested the blood
As shown in Figure 1, blood glucose was markedly higher in the premenstrual phase than in the preovulatory phase (P<0.05), indicating that the reduction of estrogen may lead to increased blood glucose in humans.

2.2 Effect of estrogen on ovariectomized mice

We first established an OVX mice model (Fig.2A). In order to verify the success of the model, we used radioimmunoassay to measure serum estradiol levels. The results showed that the serum estradiol level of the OVX mice was significantly lower than that of the Sham mice (Fig. 2B). In addition, we used immunohistochemistry and Western blot to detect the expression levels of estrogen receptors ER-α and ER-β in the duodenal tissues of the two groups. The results showed that compared with mice in the Sham group, the expression of ER-α (Fig.2C, E) and ER-β (Fig.2D, F) in the duodenum tissue of the OVX mice was significantly reduced. It is indicated that after estrogen deficiency, the receptors ER-α and ER-β of estrogen in duodenum tissue are also significantly down-regulated. These results means that the model was successfully established.

We continuously observed and recorded the bodyweight of the mice after the operation. The results showed that the weight of the two groups of mice showed an upward trend two weeks after the operation, but there was no significant difference in the weight change at 1 week after the operation. From the 8th day, the body weight of the OVX mice was significantly higher than that of the Sham mice (Fig.2G). Two weeks after the operation, we randomly dissected the two groups of mice to observe the fat in the abdominal cavity. Similarly, the abdominal fat of the OVX mice was significantly increased (Fig.2I). In order to explore whether the OVX mice caused obesity through estrogen affecting glucose tolerance, we tested the changes in blood glucose of the two groups of mice through the OGTT experiment. Consistent with human research experiments, the blood glucose level in the OGTT experiment of the OVX mice was significantly higher than that of the Sham mice (Fig.2H), which indicated that the glucose tolerance of the ovariectomized mice was reduced and the blood glucose was significantly increased.

2.3 Effect of estrogen on the absorption of glucose in the duodenum

The above results have confirmed that with the level of estrogen is decreased (Fig.2B) in OVX mice, the expression of ER-α and ER-β in the intestinal mucosa is significantly reduced (Fig. 2C, F), so whether the reduction of estrogen will affect the absorption of intestinal glucose? We used Ussing chamber technology to detect the changes of short-circuit current (Isc) in duodenum. After glucose stimulation, the changes of Isc in OVX mice was significantly lower than that in Sham group (Fig.3A), suggesting that estrogen deficiency results in a decrease in glucose absorption. In order to find possible mechanisms that decreased glucose absorption, we examined the effects of estrogen on duodenal glucose transporter of mice. Firstly, the expression of SGLT1 and GLUT2 in duodenum of mice were detected by immunohistochemistry. The results showed that the expression of SGLT1 (Fig.3B) and GLUT2 (Fig.3C) in duodenum of mice in OVX mice was significantly lower than that in Sham mice.
Further, the effect of estrogen on duodenal glucose transport protein was confirmed by in vitro cell experiments. We tested by Western-blot the expression of SGLT1 and GLUT2 in SCBN cells after 17β-Estradiol treatment for 24 and 48h. Compared with the control, the expression of SGLT1 and GLUT2 in SCBN cells was significantly increased after 17β-Estradiol treatment (Fig.4A, B), which indicated that estrogen promotes the expression of glucose transporters.

In order to further screen the functional receptors binding to estrogen, we silenced ER-α or ER-β receptor with lentivirus in SCBN cells (Fig.4C, D). It was found that after silencing ER-α expression, the up-regulation effect of estrogen on the protein expression of SGLT1 and GLUT2 was significantly reversed, while Silencing ER-β expression had little effect on it, and the difference was not statistically significant (Fig. 4E). This result indicated that ER-α shRNA, but not ER-β shRNA, inhibited 17β-estradiol treatment-induced SGLT1 and GLUT2 expression increase.

2.4 Effect of PKC pathway on estrogen-mediated glucose absorption in duodenum

At present, studies have shown that estrogen is involved in the activation of PKC signaling pathway and is an important starting valve for PKC activation[15-17]. Does PKC participate in the regulation of estrogen on intestinal glucose absorption? We further explored the role of PKC signaling pathway. As shown in Fig.5A, after treating SCBN cells with 17β-estradiol, the expression of p-PKC decreased significantly, and as the administration time increased, the effect became more pronounced, suggesting that estrogen inhibits PKC signaling pathway. Subsequently, the effect of PKC pathway on duodenal glucose transporter was observed by PKC agonist PMA and inhibitor Gö6976. The results showed that the expression of SGLT1 and GLUT2 decreased significantly in SCBN cells stimulated by agonist PMA (Fig.5B, C), while the expression of SGLT1 and GLUT2 increased significantly after treatment with inhibitor Gö6976 (Fig.5D, E), indicating that PKC pathway negatively regulates the expression of glucose transporters SGLT1 and GLUT2. we further studied the combined action of estrogen and PMA or Gö6976 to glucose transporter. The results showed that compared with estrogen alone, the co-treatment of PMA and estrogen could significantly reverse the promoting effect of estrogen on the expression of SGLT1 and GLUT2, while the effect of estrogen on the expression of SGLT1 and GLUT2 was synergistically promoted by Gö6976(Fig. 5F, G).

Finally, we investigated the effect of ER-α or ER-β on the PKC pathway. 17β-estradiol administrated silenced ER-α or ER-β SCBN cells, which found that estrogen did not reduce the expression of p-PKC in silenced ER-α SCBN cells compared with control treated by 17β-Estradiol, but expression of p-PKC in silenced ER-β SCBN cells was the same as that control treated with 17β-Estradiol, that is, estrogen inhibited the expression of p-PKC in silenced ER-β SCBN cells and control cells treated by 17β-Estradiol. Moreover, PMA increased PKC phosphorylation in ER-α-silenced SCBN cells, after adding PKC Inhibitor Gö6976, PKC phosphorylation induced by PMA decreased significantly (Fig.5H). Therefore, the above results suggested that estrogen inversely regulates the expression of p-PKC, where ER-α played a crucial role.
Discussion

In this study, we investigated the effects of estrogen on glucose transporters in human, mice and SCBN cells. We found that the lack of estrogen can cause weight gain, abdominal fat increase, and blood glucose increase. This confirms previous studies that estrogen deficiency in postmenopausal women or OVX animals are associated with obesity[18, 19], and the increase of abdominal fat consistent with the role of estrogen in regulating the distribution of fat[20]. Traditionally, obesity is related to excessive sugar intake[21, 22], and dietary fructose, compared with glucose, can lead to dyslipidemia, insulin resistance and increased hepatic de novo lipogenesis[23]. However, we found that blood glucose is increased in OVX mice, on the contrary, the absorption of glucose in the duodenum is reduced, which broke the normal concept.

So what is the reason for this result?

As we all know, the pancreas plays a pivotal role in regulating the digestion of nutrients and maintaining energy homeostasis. It is the main organ that regulates the body's blood glucose. The stable maintenance of blood glucose levels is achieved by the opposite action of glucagon and insulin, and previous studies have shown that estrogen participates in the regulation of pancreatic endocrine by affecting cytosolic Ca\(^{2+}\) concentration, that is, enhancing the release of insulin and reducing the release of glucagon [24]. Animal experiments also show that islets isolated from OVX mice release less insulin in response to glucose than islets isolated from intact mice[25], and OVX mice can also cause an increase in glucagon concentration[26]. Therefore, the lack of estrogen may cause pancreatic islet dysfunction and lead to increased blood sugar. Although earlier studies have found that in menopausal women, insulin secretion does not seem to be different from that of pre-menopausal women. This may be due to producing less insulin and eliminating it more slowly—thus compensating for reduced secretion and maintaining insulin at premenopausal levels[27]. However, this effect needs further research, because postmenopausal women still need to consider all potential confounding factors. In addition, in OVX mice, does the lack of estrogen also cause the secretion of other glucose-increasing hormones (such as: cortisol, thyroid hormone, growth hormone, catecholamines) to increase and then to increase blood sugar?

In previous animal studies, researchers found that compared with females with intact gonads, ovariectomy reduced the baseline and post-stress concentrations of corticosterone[28-30], and reduced the secretion of thyroid hormone [31, 32]and growth hormone[33, 34], while ovariectomy significantly increased kidney and plasma catecholamine levels[35, 36], and these reactions can be reversed by exogenous estradiol supplementation. However, whether the increase of catecholamines after ovariectomy promotes the increase of blood sugar and its mechanism of action require further experimental studies.

In addition to islet dysfunction and other hormones secretion, we also considered another most important reason why estrogen deficiency causes blood glucose increase in ovariectomized mice: insulin
resistance.

Both preclinical studies and clinical studies have shown that decreased or lack of estrogen can lead to insulin resistance[37, 38]. According to the study of different mechanisms, we found that there are two main possible mechanisms of insulin resistance in OVX mice: one is primary insulin resistance mediated by estrogen deficiency, and the other is secondary insulin resistance due to fat deposition after estrogen reduction.

In insulin-responsive tissues (such as adipocytes), ERα plays a pivotal role in regulating insulin signal transduction, GLUT4 translocation, glucose uptake and GLUT4 expression[25]. However, in OVX mice, lack of estrogen greatly weakens the effect of ERα, which leads to weakened insulin signal, reduced GLUT4 expression and glucose uptake. Besides, estrogen deficiency also affects calcium homeostasis[39], for example, Tsang et al. found that ovariectomy can increase [Ca^{2+}]i by increasing the expression of L-type voltage-gated calcium channel (VGCC) [40]. In insulin-responsive cells, disturbance of Ca^{2+} homeostasis affects gene regulation and insulin signal transduction, and interferes with the translocation or fusion of GLUT4 to the cell membrane [41, 42]. Ultimately, these abnormalities will cause the target tissue cells to be insensitive to insulin.

On the other hand, in the visceral fat of OVX mice, the interaction of adipose triglyceride lipase and comparative gene recognition-58 increased, and the content of peripheral lipoproteins decreased, which contributed to increased levels of glycerol and non-esterified fatty acids, and increased visceral fat mass and lipid accumulation [43]. As fat storage increase, more free fatty acids (FFA) are released, leading to increased mobilization of FFA and consequently to increased FFA oxidation in muscle and liver. When FFA are used as an alternative energy source, muscle consumption of glucose will be reduced, and liver glucose production will increase with the increase of FFA oxidation, leading to hyperglycemia and impaired glucose tolerance[44].

The previous study found that the nerve growth factor (NGF) receptor-p75 neurotrophin receptor (p75NTR), a regulator of glucose uptake and insulin resistance, differentially regulates Rab5 and Rab31, resulting in decrease in GLUT4 plasma membrane translocation, which led to a decrease in insulin-stimulated glucose uptake. Furthermore, p75NTR knockout mice shows higher insulin sensitivity on normal diet[45, 46]. Our further found that p75 NGF receptor measured by western blotting was significantly up-regulated in OVX mice (supplementary Fig. 1), which indicated that ovariectomy caused insulin resistance, and this conclusion is consistent with previous studies[47]. Interestingly, this response can be reversed by estrogen supplementation[48, 49].

In this study, we found that estrogen can participate in regulating the absorption of glucose in the duodenum by inhibiting the PKC signaling pathway, and the estrogen receptor ER-α plays a crucial role in this process (Fig.6). Impaired glucose tolerance and increased insulin resistance in ER-α-deficient mice [50] also supports our findings that ER-α plays a crucial role in glucose homeostasis and metabolism. At the same time, we also found that PKC negatively regulates the role between estrogen
and glucose transporter. Therefore, we believe that the decrease in the absorption of glucose in the duodenum may be due to the decreased secretion of estrogen, which can’t inhibit the PKC signaling and further inhibit the expression of SGLT1 and GLUT2.

In conclusion, this study demonstrated for the first time that estrogen regulates duodenal glucose absorption through the effect of estrogen receptor-α on glucose transporters, and inhibits PKC signaling to regulate this process. This provides us with a new theoretical basis for further research and prevention of obesity and other diseases, and also provides a basis for the clinical application of estrogen drugs.

**Abbreviation**

ER: estrogen receptor; SGLT1: Sodium-Glucose Cotransporter 1; GLUT2: glucose transporter 2; OVX: ovariectomized; Isc: short-circuit current; OGTT: oral glucose tolerance test;

**Declarations**

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**Author contributions**

WS and JD conceived of the study; RX and JX designed the experiments; WS, JD, QD, CC, QL, XY, JL and ZJ and performed the experiments; JX and MC performed analysis and interpretation of data; RX and JX wrote the manuscript; RX, MC and JX critically reviewed the manuscript. All authors read and approved the final manuscript.

**Conflicts of Interest**

The authors declare that they have no competing interests.

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**Figures**
Figure 1

Effect of estrogen on human blood glucose. OGTT test was used to detect the blood glucose level during menstruation and ovulation. (*P<0.05, vs menstrual period, n=12)

Image not available with this version

Figure 2
Effect of estrogen in OVX and Sham mice. (A) After oophorectomy and sham operation, the ovaries were observed. (B) The serum estrogen levels of the two groups of mice (n=10) were measured by radioimmunoassay two weeks after operation. Immunohistochemistry to analyze the distribution and location of ER-α (C) and ER-β (D) in duodenal tissue. Western blot was used to analyze the protein expression changes of ER-α (E) and ER-β (F) in the duodenum of mice, and GAPDH was used as a protein control. The daily weight changes were observed and recorded from the second day to 2 weeks after operation (G). Changes of blood glucose (H) and abdominal fat (I) in mice 2 weeks after operation (*P<0.05, **P<0.01, ***P<0.001, compared with control, n=5).

**Image not available with this version**

**Figure 3**

Effect of estrogen on duodenal glucose absorption in mice. (A) Using chamber technology to detect the changes of Isc caused by the duodenal glucose absorption. The expressions of SGLT1 (B) and GLUT2 (C) in the duodenum of the two groups were detected by immunohistochemistry (*P<0.05, **P<0.01, compared with control, n=5).
Figure 4

Effect of estrogen on glucose absorption in duodenal epithelial cells (SCBN). The expressions of SGLT1 (A) and GLUT2 (B) were detected by Western blot in SCBN cells stimulated by estrogen for 24 and 48 hours. Western blot to verify the expression of ER-α(C) and ER-β(D) in SCBN cells after virus transfection, then detect the effect of 17β-estradiol on the proteins expression of SGLT1 and GLUT2 in it(E). (*P<0.05, **P<0.01, compared with control, n=4)
Figure 5

Effect of PKC pathway on estrogen-mediated glucose absorption in SCBN cells. (A) Changes of p-PKC in SCBN cells stimulated by estrogen. Effect of PKC agonist PMA on the expression of SGLT1 (B) and GLUT2 (C). Effect of PKC Inhibitor GÖ6976 on the expression of SGLT1 (D) and GLUT2 (E). Effect of estrogen combined with PMA or GÖ6976 on the changes of SGLT1 (F) and GLUT2 (G), respectively. (H)
After silencing ER-α and ER-β in SCBN cells, changes of p-PKC stimulated by 7β-estradiol combined with PMA or GÖ6976. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, &P<0.05, compared with control, n=4)

**Figure 6**

**Regulatory mechanism of estrogen by ER-α on intestinal glucose transporter.** Estrogen binds to the plasma membrane-located ER-α, decreases PKC activation and further increases SGLT1 and GLUT2 expression, which is indicated in blue. PMA, an agonist of PKC, inhibits the expression of SGLT1 and GLUT2, which is indicated in green. PKC inhibitor GÖ6976 promotes the expression of SGLT1 and GLUT2, which is indicated in grey.

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