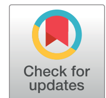

Estrogen Improves Insulin Sensitivity and Suppresses Gluconeogenesis via the Transcription Factor Foxo1

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Premenopausal women exhibit enhanced insulin sensitivity and reduced incidence of type 2 diabetes (T2D) compared with age-matched men, but this advantage disappears after menopause with disrupted glucose homeostasis, in part owing to a reduction in circulating 17β-estradiol (E_2). Fasting hyperglycemia is a hallmark of T2D derived largely from dysregulation of hepatic glucose production (HGP), in which Foxo1 plays a central role in the regulation of gluconeogenesis. Here, we investigated the action of E_2 on glucose homeostasis in male and ovariectomized (OVX) female control and liver-specific Foxo1 knockout (L-F1KO) mice and sought to understand the mechanism by which E_2 regulates gluconeogenesis via an interaction with hepatic Foxo1. In both male and OVX female control mice, subcutaneous E_2 implant improved insulin sensitivity and suppressed gluconeogenesis; however, these effects of E_2 were abolished in L-F1KO mice of both sexes. In our use of mouse primary hepatocytes, E_2 suppressed HGP and gluconeogenesis in hepatocytes from control mice but failed in hepatocytes from L-F1KO mice, suggesting that Foxo1 is required for E_2 action on the suppression of gluconeogenesis. We further demonstrated that E_2 suppresses hepatic gluconeogenesis through activation of estrogen receptor (ER)α–phosphoinositide 3-kinase–Akt–Foxo1 signaling, which can be independent of insulin receptor substrates 1 and 2 (Irs1 and Irs2), revealing an important mechanism for E_2 in the regulation of glucose homeostasis. These results may help explain why premenopausal women have lower incidence of T2D than age-matched men and suggest that targeting ERα can be a potential approach to modulate glucose metabolism and prevent diabetes.

The prevalence of type 2 diabetes (T2D) has shown sex disparities, with a reduced incidence in women (1). Both clinical and animal studies show a strong correlation between estrogen deficiency and metabolic dysfunction (2,3). The reduction of estrogen in postmenopausal women accelerates the development of insulin resistance and T2D (4). Clinical trials of estrogen replacement therapy in postmenopausal women demonstrated an amelioration of insulin resistance and reductions of plasma glucose level and incidence of T2D (5,6). However, the potential risk of breast cancer and stroke upon estrogen therapy underscores 17β-estradiol (E_2) as a therapeutic agent (7,8). Thus, understanding of tissue-specific E_2 action and its molecular mechanism in metabolic regulation is required for the development of targeted estrogen mimics conveying metabolic benefit without side effects.

The maintenance of glucose homeostasis depends on glucose uptake in muscle and adipose tissue and glucose production through gluconeogenesis and glycogenolysis in liver (9). Estrogen reduces glucose level, but it is thought to be derived from the promotion of glucose uptake in muscle (10). However, some studies have also indicated that estrogen suppresses glucose production in the liver (11–13) and that impairing the estrogen receptor (ER)-mediated signaling results in hepatic insulin resistance and hyperglycemia with elevated hepatic glucose production

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(HGP) (14,15). These lines of evidence underscore the importance of estrogen signaling in the liver in control of glucose homeostasis; yet, the liver-specific action of estrogen on HGP and its mechanism remain unclear.

Foxo1, a member of *O*-class forkhead transcription factor, is a predominant regulator for HGP, promoting gene transcription of the rate-limiting gluconeogenic enzyme glucose-6-phosphatase (*G6pc*) (16,17). Foxo1 is suppressed by insulin through the activation of Akt via the insulin receptor substrate (Irs)1 and Irs2-associated phosphoinositide 3-kinase (PI3K) (18,19). In a T2D mouse model, insulin fails to activate Akt and suppress Foxo1, resulting in enhanced HGP and hyperglycemia; this can be prevented by deletion of hepatic Foxo1 (20). In this study, we investigated the effect of E_2 on the maintenance of glucose homeostasis and the involvement of hepatic Foxo1 in mice of both sexes. We further examined E_2 regulatory mechanisms for HGP and gluconeogenesis in primary hepatocytes. In particular, we tested the hypothesis that hepatic Foxo1 plays important roles in the E_2 action of suppressing hepatic gluconeogenesis through ER-associated Akt signaling.

RESEARCH DESIGN AND METHODS

Animals

Animal protocols were approved by the institutional animal care and use committee at Texas A&M University. Liver-specific Foxo1 knockout (L-F1KO) mice were generated by breeding floxed Foxo1$^{L/L}$ mice with albumin-Cre mice as previously described (21). The Cre recombinase induced by the mouse albumin enhancer/promoter shows no influence on animal performance, as previously reported, and either the floxed-Foxo1$^{L/L}$ or albumin-Cre littermates were used as control mice in our studies (22,23). Eight- to 12-week-old control (Foxo1$^{L/L}$) and L-F1KO (albumin-Cre$^{+/-}$:: Foxo1$^{L/L}$) mice on a mixed genetic background of C57BL/6J and 129/Sv were fed a standard chow diet (54% calories from carbohydrate, 14% from fat, and 32% from protein and 3.0 kcal/g) (Envigo Teklad Diet) ad libitum.

Ovariectomy and E_2 Replacement

Control and L-F1KO mice were randomly assigned to experimental groups (n = 4–7). All mice were subjected to the subcutaneous implantation of placebo or E_2 pellet (0.05 mg/pellet, 60-day release) (Innovative Research of America, Sarasota, FL). Female mice underwent a bilateral ovariectomy (OVX) surgery (except for intact control mice) at the time of pellet implantation.

Metabolic Analyses

Blood glucose levels were measured using a glucometer (Bayer, Whippany, NJ). Glucose tolerance tests (GTTs) and pyruvate tolerance tests (PTTs) were performed in mice fasted for 16 h. Insulin tolerance tests (ITTs) were conducted in mice fasted for 5 h. Serum samples were collected from mice fasted for 16 h by cardiac puncture for determination of serum insulin, glucagon, and other hormone levels by Bio-Plex mouse diabetes immunoassay (Bio-Rad, Hercules, CA) and serum E_2 concentration by an ELISA kit (Cayman, Ann Arbor, MI). Liver glycogen content was measured from mice fasted 16 h as previously described (24).

Cell Cultures and HGP Assay

Primary hepatocytes were isolated from 8- to 16-week-old mice and cultured in DMEM with 10% FBS as previously described (21). Cells were serum starved for 6 h before treatment with 100 nmol/L E_2 and a variety of signal transduction inhibitors, which were applied 30 min prior to E_2. For HGP assay, freshly isolated hepatocytes were cultured in DMEM with 2% FBS. After 3 h of attachment, cells was cultured in HGP buffer (120 mmol/L NaCl, 5.0 mmol/L KCl, 2.0 mmol/L $CaCl_2$, 25 mmol/L $NaHCO_3$, 2.5 mmol/L KH_2PO_4, 2.5 mmol/L $MgSO_4$, 10 mmol/L HEPES, 0.5% BSA, 10 mmol/L sodium DL-lactate, and 5 mmol/L pyruvate, pH 7.4) and treated with chemicals. Culture medium was collected to determine glucose production using an Amplex Red Glucose Assay kit (Invitrogen, Carlsbad, CA). Total HGP, glycogenolysis, and gluconeogenesis were determined and calculated as previously described (25).

Quantitative Real-time PCR

RNA was extracted with Trizol reagent (Invitrogen) and used for cDNA synthesis via an iScript cDNA Synthesis kit (Bio-Rad). Gene expression was measured with SYBR Green Supermix in real-time PCR (Bio-Rad) using primers as previously described and cyclophilin gene as an internal control (21).

Protein Immunoblotting

An equal amount of protein was resolved in SDS-PAGE and transferred to nitrocellulose membrane for Western blot. Antibodies for Foxo1, phosphorylated Foxo1 at Ser^{253}, Akt, phosphorylated Akt at Ser^{473}, Pck1, and GAPDH were purchased from Cell Signaling Technology. Protein densitometry was performed and analyzed using ImageJ as previously described (21).

Statistical Analyses

Data were analyzed by one-way or two-way ANOVA to determine the significance of the model as appropriate. The interaction between E_2 and L-F1KO was analyzed in two-way ANOVA to determine the dependency. Differences between groups were determined by Tukey post hoc test or Student t test as appropriate. $P < 0.05$ was considered indicative of statistical significance. Results are presented as means $\pm$ SEM.

RESULTS

E_2 Regulates Glucose Homeostasis Depending on Hepatic Foxo1 in Male Mice

We determined the role of estrogen in control of glucose homeostasis and its dependence on hepatic Foxo1.

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Generally, intact female mice exhibited a 16% lower fasting glucose level compared with male mice (intact female 51 ± 2.8 mg/dL vs. male: 61 ± 2.3 mg/dL, P = 0.03) (Fig. 1*A* and *C*). In female mice, OVX mice had a 22% increase in fasting glucose compared with intact females (intact 51 ± 2.8 mg/dL vs. OVX 62.4 ± 2.2 mg/dL, $P < 0.01$) (Fig. 1*A*). E_2 reduced glucose levels by 21% in OVX control females (OVX + placebo 62.4 ± 2.2 mg/dL vs. OVX + E_2

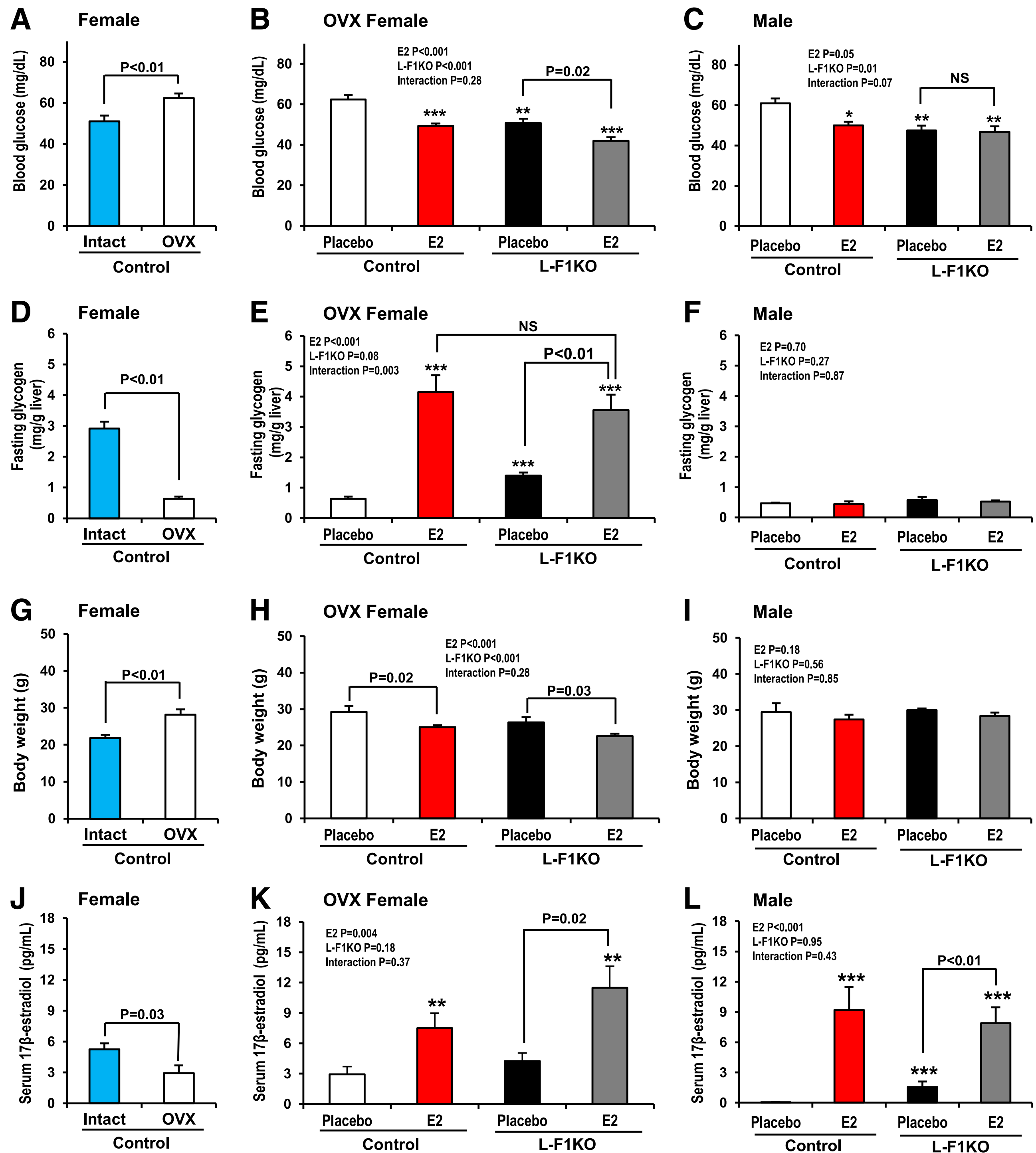

Figure 1—Estrogen regulates glucose homeostasis in mice. *A–C*: Blood glucose level in control and L-F1KO mice of both sexes fasted for 16 h overnight. Fasting glucose was measured every week, and the data show the most representative measurement. *D–F*: Glycogen content in the liver of mice fasted for 16 h overnight. Glycogen content was normalized by weight of liver. *G–I*: Body weight at 8 weeks after E_2 implantation in control and L-F1KO mice of both sexes fasted overnight. *J–L*: Serum E_2 level at 8 weeks after E_2 implantation in mice fasted overnight. All data are expressed as the mean ± SEM. For females, n = 4–7, *$P < 0.05$ vs. OVX control mice with placebo. For males, n = 4–5, *$P < 0.05$ vs. control mice with placebo. NS, not significant ($P > 0.05$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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49.4 ± 1.2 mg/dL, $P < 0.001$) and almost restored glucose levels to those of intact mice (Fig. 1*A* and *B*). In control male mice, E_2 decreased glucose levels by 16% (placebo 61.0 ± 2.3 mg/dL vs. E_2 51.0 ± 2.0 mg/dL, $P = 0.03$) (Fig. 1*C*). Deficiency of hepatic Foxo1 decreased fasting glucose by 19% in OVX females and by 23% in males (Fig. 1*B* and *C*). In L-F1KO mice, E_2 led to a further 17% reduction of glucose levels in OVX females but failed to reduce glucose levels in males (Fig. 1*B* and *C*). These results indicate that hepatic Foxo1 is required for E_2 to regulate glucose homeostasis in males rather than females.

To explain the sex disparity in glucose levels of L-F1KO mice in response to E_2, we measured fasting glycogen content in the liver. Intact female mice showed a sevenfold higher liver glycogen than that of males, indicating a higher capacity of females to store glycogen and/or prevent glycogenolysis in the liver than males (Fig. 1*D* and *F*). In control females, OVX reduced liver glycogen by 80% ($P < 0.01$), but this effect of OVX was reversed by E_2 ($P < 0.01$) (Fig. 1*D* and *E*). L-F1KO increased liver glycogen by 2.2-fold in OVX females, and E_2 further increased liver glycogen by 2.5-fold in OVX L-F1KO females (Fig. 1*E*); however, the effect of L-F1KO is masked by E_2 when E_2 is abundantly available. Collectively, the sex differences in liver glycogen may help explain the sex disparity in glucose homeostasis in response to E_2.

Compared with intact female mice, OVX increased body weight by 40% (Fig. 1*G*), while E_2 reduced body weight of OVX females by 14% in both control and L-F1KO mice (Fig. 1*H*). Body weight was unaffected by E_2 in male mice (Fig. 1*I*). Deletion of hepatic Foxo1 had no effect on body weight in both sexes (Fig. 1*G*–*I*). OVX mice exhibited a 44% lower serum E_2 level compared with intact females owing to absence of ovarian estrogen production (Fig. 1*J*). E_2 implant significantly increased serum E_2 levels of control and L-F1KO mice in both sexes ($P < 0.05$) (Fig. 1*K* and *L*). Interestingly, in males, serum E_2 concentration was undetectable in control mice, but L-F1KO males exhibited a detectable serum E_2 value at 1.56 ± 0.53 pg/mL (Fig. 1*L*).

Hepatic Foxo1 Is Necessary for Estrogen to Improve Insulin Sensitivity and Suppress Gluconeogenesis in Mice

We next examined the role of hepatic Foxo1 in E_2 action on insulin sensitivity and gluconeogenesis in mice. Surgical removal of the ovaries impaired glucose clearance after intraperitoneal injection of glucose (Fig. 2*A* and *D*). Either E_2 implant or deletion of liver Foxo1 resulted in an improved glucose tolerance in both sexes, but there were no additive effects of these two in L-F1KO mice (Fig. 2*B*–*F*). We examined whether estrogen-lowered fasting glucose is derived from suppression of gluconeogenesis, as determined by PTT. OVX had impaired pyruvate tolerance compared with control intact females ($P < 0.05$) (Fig. 2*G* and *J*), while E_2 suppressed gluconeogenesis in both sexes of control mice ($P < 0.05$) (Fig. 2*H*–*L*). L-F1KO mice displayed a decrease in gluconeogenesis in both sexes ($P < 0.05$); however, the effect of E_2 was abolished in L-F1KO mice (Fig. 2*H*–*L*). OVX impaired insulin sensitivity as determined by ITT (Fig. 2*M* and *P*). E_2 and L-F1KO improved ITT in both sexes but had no additive effect (Fig. 2*N*–*R*). Taken together, E_2 improves insulin sensitivity and suppresses gluconeogenesis in a Foxo1-dependent manner.

We measured mRNA levels of genes responsible for insulin signaling and gluconeogenesis. The OVX surgery suppressed hepatic *Irs2* mRNA expression by 40% and induced *G6pc* and phosphoenolpyruvate carboxykinase 1 (*Pck1*) expressions by 22% and 42%, respectively, in control females ($P < 0.05$); however, E_2 induced *Irs2* by 35% and suppressed *G6pc* and *Pck1* by 22% and 30% in OVX control females (Fig. 3*A* and *B*). E_2 also induced *Irs2* by 50% and suppressed *G6pc* by 24% in male mice. The liver of L-F1KO mice displayed a reduction of *Irs1* expression by 45% in OVX females and 20% in males independent of E_2 treatment (Fig. 3*B* and *C*). Hepatic expression of *G6pc* was reduced by 46–48% in both sexes upon deletion of liver Foxo1 ($P < 0.05$) (Fig. 3*B* and *C*). However, E_2 failed to have the inhibitory effect on *G6pc* and *Pck1* expressions in both sexes of L-F1KO mice (Fig. 3*B* and *C*).

Levels of metabolic hormones were determined in the serum from mice fasted for 16 h. OVX mice exhibited increases in leptin and plasminogen activator inhibitor 1 levels by 3.0- and 1.3-fold, respectively, compared with intact female mice, while E_2 implant decreased levels of leptin and plasminogen activator inhibitor 1 by 56% and 30% in OVX mice ($P < 0.05$) (Fig. 3*D* and *E*). In male mice, deletion of liver Foxo1 increased glucagon and ghrelin levels by 5.1- and 2.6-fold, respectively ($P < 0.05$) (Fig. 3*F*). E_2 increased ghrelin level by 1.8-fold in control males ($P < 0.05$) (Fig. 3*F*). Although sex differences exist in the release of metabolic hormones, the increased glucagon and ghrelin levels, as well as decreased leptin level, may be a result of lower blood glucose upon E_2 stimulation and ablation of Foxo1, which suggests a feedback regulation in glucose homeostasis.

Hepatic Foxo1 Is Required for Estrogen to Suppress HGP and Gluconeogenesis in Primary Hepatocytes

We next assessed E_2 action on hepatic glucose metabolism in primary hepatocytes. L-F1KO hepatocytes exhibited a 32% lower HGP after 1 h culture in assay buffer compared with control cells ($P < 0.05$) (Fig. 4*A*). E_2 decreased HGP by 14% and 20% after 3- and 6-h culture in control cells, respectively ($P < 0.05$), but failed to suppress HGP in L-F1KO cells (Fig. 4*A*). We further divided HGP into gluconeogenesis and glycogenolysis in response to E_2. Although E_2 decreased glycogenolysis by 25% in control hepatocytes ($P < 0.05$), the contribution of glycogen breakdown to total HGP was much smaller than that of gluconeogenesis (Fig. 4*B*). Thus, 18% reduction of gluconeogenesis by E_2 mainly contributed to suppression of HGP in control cells ($P < 0.05$) (Fig. 4*B*). Consistently, E_2 reduced expressions of *G6pc* by 25% and *Pck1* by 30% in

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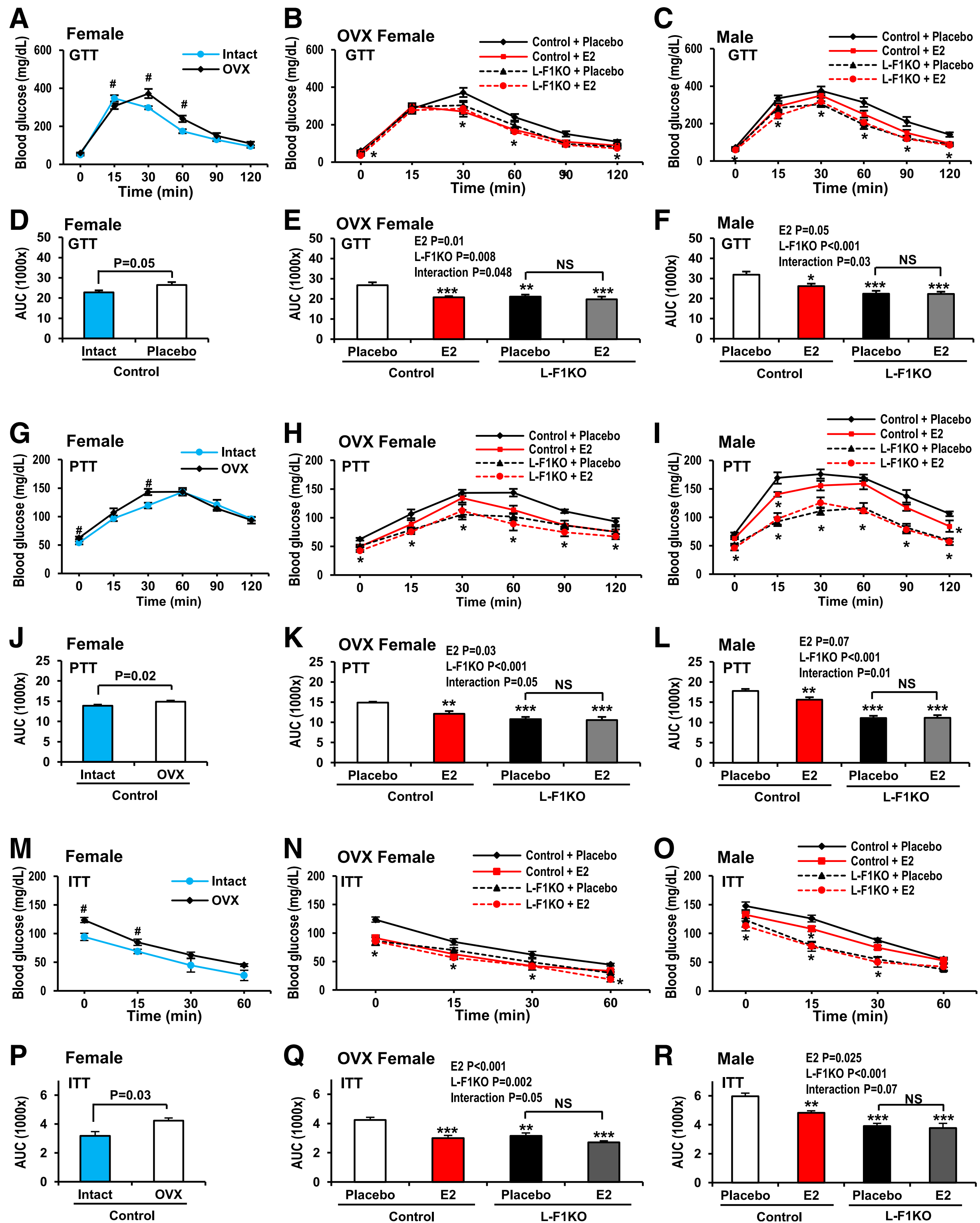

Figure 2—Estrogen improves insulin sensitivity and suppresses gluconeogenesis in mice. *A–C*: GTT was performed in control and L-F1KO mice of both sexes fasted for 16 h overnight. Glucose was administered at 2 g/kg body wt of mice by intraperitoneal injection after 16 h overnight fasting, and glucose level was measured at indicated time points. *D–F*: Area under the curve (AUC) of the GTT displayed in *A–C*, respectively. *G–I*: PTT was performed on mice fasted for 16 hours overnight. Pyruvate was administered at 2 g/kg body wt by intraperitoneal injection after overnight fasting, and glucose level was measured at indicated time points. *J–L*: Area under the curve of the PTT showed in *G–I*, respectively. *M–O*: ITT was performed on mice fasted for 5 h. Insulin was administered at 2 units/kg body wt by intraperitoneal injection, and glucose level was measured at indicated time points. *P–R*: Area under the curve of the ITT shown in *M–O*, respectively. All data are expressed as mean ± SEM. For females, n = 4–7, *$P < 0.05$ vs. OVX control mice with placebo. n = 4–7, #$P < 0.05$ vs. intact mice. For males, n = 4–5, *$P < 0.05$ vs. control mice with placebo. NS, not significant ($P > 0.05$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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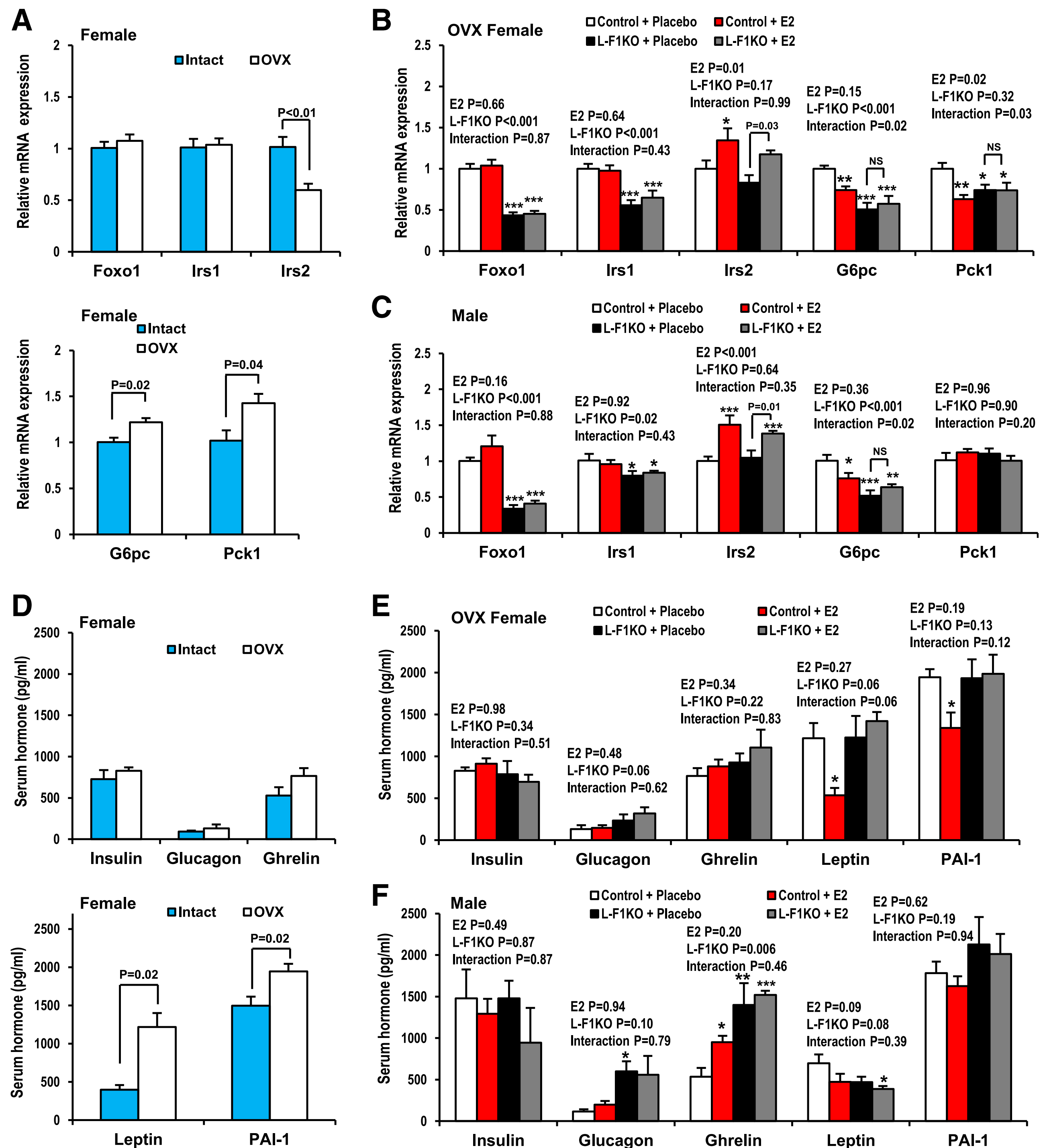

Figure 3—Estrogen suppresses expression of gluconeogenic genes in liver and influences serum hormone levels associated with glucose metabolism in mice. *A–C*: Relative mRNA expressions in the liver of control and L-F1KO mice of both sexes fasted for 16 hours overnight after 8 weeks of pellet implantation. *D–F*: Serum hormone levels in mice fasted for 16 h overnight. Blood samples were collected by cardiac puncture in euthanized mice. All data are expressed as the mean ± SEM. For females, n = 4–7, *P < 0.05 vs. OVX control mice on placebo. For males, n = 4–5, *P < 0.05 vs. control mice on placebo. *P < 0.05; **P < 0.01; ***P < 0.001.

control hepatocytes ($P < 0.05$) (Fig. 4*C*). L-F1KO hepatocytes showed reductions of *G6pc* and *Pck1* expressions by 55% compared with control cells ($P < 0.05$); however, E_2 failed to further suppress these genes' expression in L-F1KO cells (Fig. 4*C*).

Akt phosphorylates Foxo1 at Ser^{253}, thus promoting Foxo1 nuclear export and/or degradation (26). E_2 increased phosphorylation of Akt and Foxo1 by 1.7- and 1.5-fold, respectively. The total Foxo1 degradation was unnoticeable by E_2 treatment alone but evident by E_2

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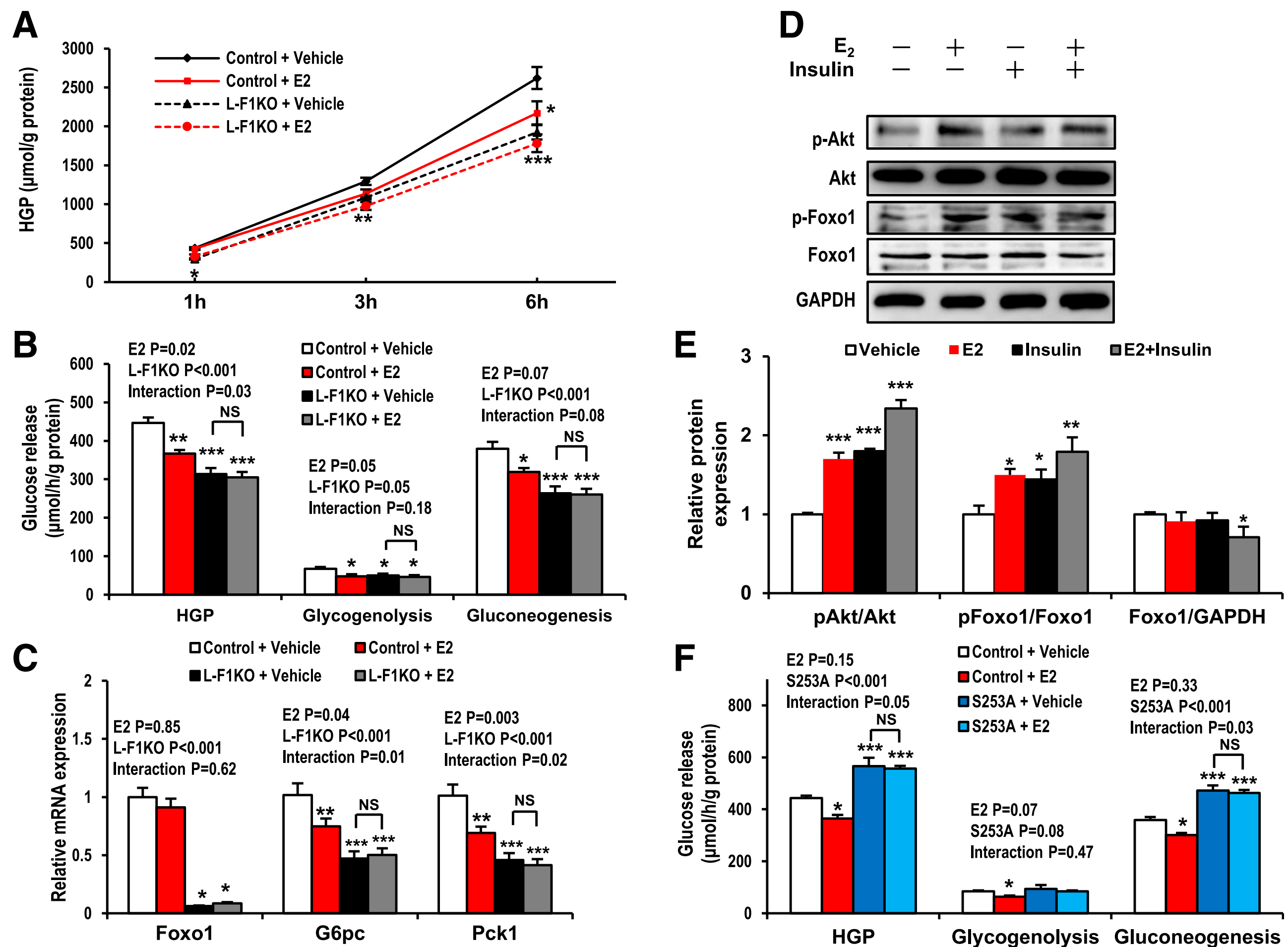

Figure 4—Estrogen suppresses HGP and gluconeogenesis depending on hepatic Foxo1 in primary hepatocytes. *A*: HGP in primary hepatocytes from control and L-F1KO mice. HGP was measured at indicated time points after 0.1 μmol/L E_2 stimulation, and normalized to total protein levels. *B*: Glucose production in primary hepatocytes from control and L-F1KO mice upon 0.1 μmol/L E_2 stimulation in the presence (HGP) or absence (glycogenolysis) of pyruvate and lactate. The difference between these two values was interpreted to represent gluconeogenesis. *C*: Relative mRNA levels of gluconeogenic genes in primary hepatocytes from control and L-F1KO mice upon 0.1 μmol/L E_2 stimulation for 3 h. *D* and *E*: Western blots (*D*) and corresponding quantification (*E*) of insulin signaling protein in hepatocytes from control mice upon 1 h stimulation of 0.1 μmol/L E_2 or 0.1 μmol/L insulin. p-, phosphorylated. *F*: Glucose production in primary hepatocytes from control and Foxo1 S253A knock-in mice (S253A) upon 0.1 μmol/L E_2 stimulation in the presence (HGP) or absence (glycogenolysis) of pyruvate and lactate (glycogenolysis). All data are expressed as the mean ± SEM. NS, not significant ($P > 0.05$). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. control + vehicle. $n = 3$ of each group.

and insulin cotreatments (Fig. 4*D* and *E*). To confirm the importance of phosphorylation of Foxo1 at Ser[253] for E_2 action, we generated Foxo1 Ser[253] knock-in mice (S253A), in which serine 253 was replaced with alanine to prevent Akt phosphorylation (K. Zhang, X. Guo, H. Yan, Y. Wu, , Q. Pan, Z. Shen, X. Li, Y. Chen, L. Li, Y. Qi, Z. Xu, W. Xie, W. Zhang, D. Threadgill, L. He, Y. Sun, M. F. White, H. Zheng, and S. Guo, unpublished observations). Hepatocytes from S253A mice exhibited increases in HGP by 31% and gluconeogenesis by 36% owing to the blockage of Foxo1 phosphorylation at Ser[253] ($P < 0.05$); meanwhile, E_2 failed to reduce HGP in S253A hepatocytes (Fig. 4*F*). These data suggested that phosphorylation of Foxo1 Ser[253] by Akt is required for E_2 in the regulation of Foxo1 activity and hepatic gluconeogenesis.

E_2 Suppresses Gluconeogenesis Dependent on PI3K-Akt Signaling but Independent of Irs1 and Irs2

We next determined whether activation of Akt signaling downstream from PI3K is necessary for E_2 in suppression of Foxo1 and gluconeogenesis in hepatocytes using Akt1/2 kinase inhibitor (Akti) or PI3K inhibitor wortmannin (Wort). Both Akti and Wort completely prevented E_2 effects on HGP, glycogenolysis, and gluconeogenesis (Fig. 5*A*), as well as E_2-suppressed *G6pc* and *Pck1* mRNA expression and Pck1 protein levels (Fig. 5*B* and *C*). Meanwhile, E_2-induced reduction of Pck1 protein level and phosphorylation of both Akt and Foxo1 were also blocked by these two inhibitors (Fig. 5*C* and *D*). Additionally, Akti alone showed increases in basal levels of *G6pc* and *Pck1* but no significant effect on HGP or gluconeogenesis in the primary hepatocytes (Fig. 5*A*–*D*).

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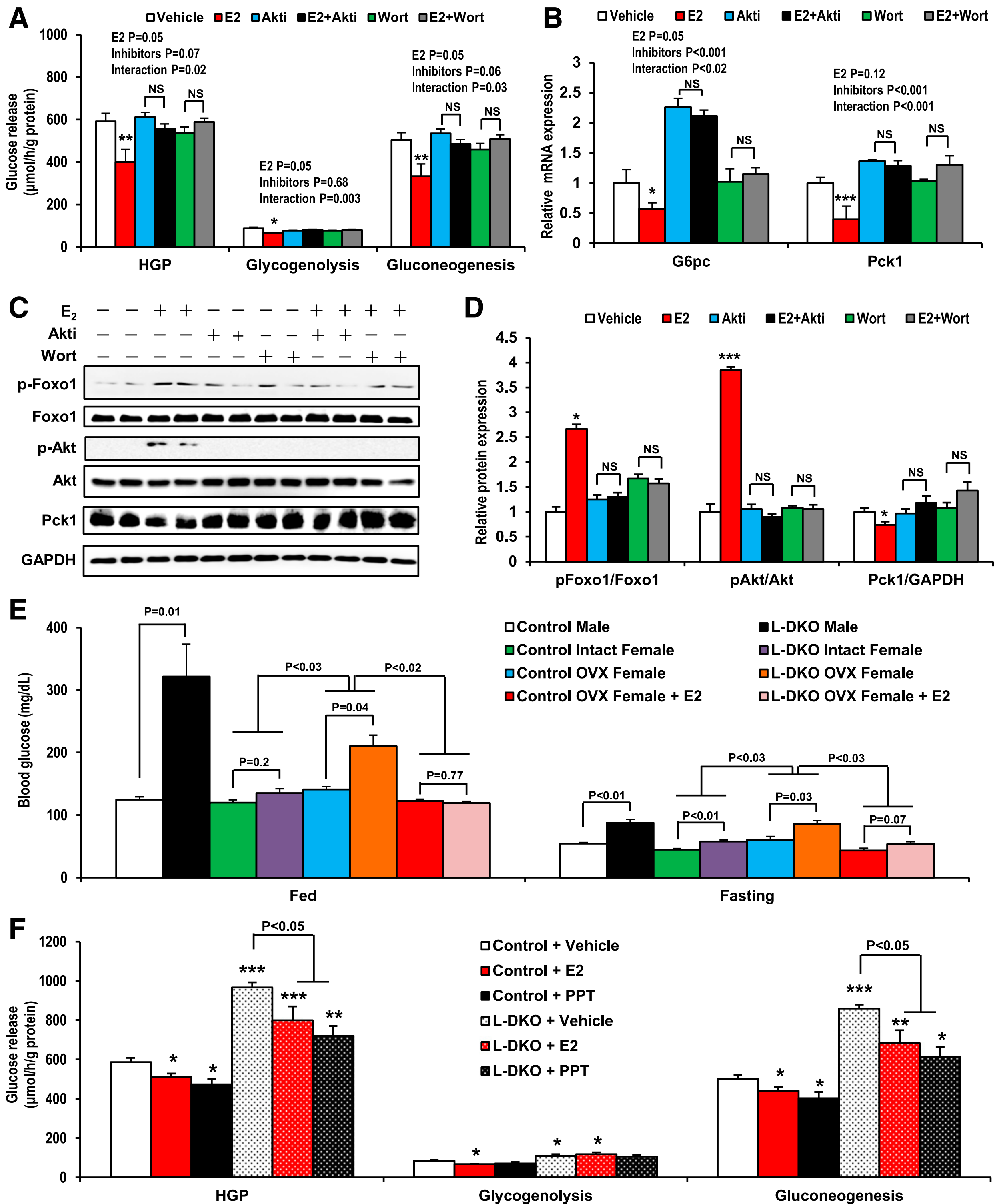

Figure 5—Activation of Akt signaling is required for estrogen to suppress gluconeogenesis. *A*: Glucose release in primary hepatocytes from control mice in the presence (HGP) or absence (glycogenolysis) of pyruvate and lactate. Cells were treated with 10 μmol/L Akti or 0.2 μmol/L Wort 30 min prior to 0.1 μmol/L E_2 stimulation. *B*: Relative mRNA levels of gluconeogenic genes in primary hepatocytes from control mice upon stimulation of 10 μmol/L Akti and 0.2 μmol/L Wort 30 min prior to 0.1 μmol/L E_2. *C* and *D*: Western blots (*C*) and corresponding quantification (*D*) of insulin-signaling protein in hepatocytes from control mice upon stimulation of 10 μmol/L Akti and 0.2 μmol/L Wort 30 min prior to 0.1 μmol/L E_2. p-, phosphorylated. *E*: Blood glucose levels of control and L-DKO mice in random-fed or fasted state upon OVX surgery and E_2 implant. Blood glucose levels were measured in littermates of male, intact female, and OVX female mice at 12 weeks of age random fed or after 16 h overnight fast. *F*: Glucose production in primary hepatocytes from control and L-DKO mice upon 0.1 μmol/L E_2 or 0.1 μmol/L ERα agonist PPT stimulation in the presence (HGP) or absence (glycogenolysis) of pyruvate and lactate. All data are expressed as the mean ± SEM. NS, not significant ($P > 0.05$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control + vehicle. $n = 3$ of each group.

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Moreover, we further determined whether Irs1 and Irs2 are required for E_2-mediated glucose homeostasis using liver-specific Irs1 and Irs2 double knockout (L-DKO) mice as we previously characterized (27). In a random-fed state, L-DKO male mice displayed hyperglycemia with 158% increased glucose level (control 124.7 ± 4.38 mg/dL vs. L-DKO 321.50 ± 51.73 mg/dL, $P = 0.01$); however, L-DKO females exhibited euglycemia (control 119.9 ± 4.37 mg/dL vs. L-DKO 135.0 ± 6.93 mg/dL, $P = 0.2$) (Fig. 5*E*). In the fasting state, L-DKO males had increased glucose level by 62% (control 54.2 ± 1.9 mg/dL vs. L-DKO 87.6 ± 5.5 mg/dL, $P < 0.01$), while L-DKO female mice only had increased glucose level by 29% (control 44.8 ± 1.65 mg/dL vs. L-DKO 57.8 ± 2.63 mg/dL, $P < 0.01$) (Fig. 5*E*). Significantly, L-DKO females developed hyperglycemia 2 weeks after OVX, with 56% increased glucose, in the random-fed state (intact L-DKO 135.0 ± 6.9 mg/dL vs. OVX L-DKO 210.3 ± 13.5 mg/dL, $P < 0.01$), while OVX only increased the glucose level by 21% in control females (intact control 119.9 ± 4.4 mg/dL vs. OVX control 136.2 ± 5.9 mg/dL, $P = 0.04$) (Fig. 5*E*). Collectively, for OVX females, random-fed L-DKO mice had a 54% increase in glucose level (control 136.2 ± 5.91 mg/dL vs. L-DKO 210.3 ± 13.5 mg/dL, $P = 0.04$) and a 43% increase in fasting glucose (control 60.4 ± 5.36 mg/dL vs. L-DKO 86.0 ± 4.89 mg/dL, $P = 0.03$) compared with control mice (Fig. 5*E*). Moreover, subcutaneous implantation of E_2 significantly decreased blood glucose of both control and L-DKO OVX mice in both fed and fasting states and totally abolished the effect of L-DKO on glucose level (Fig. 5*E*). Additionally, in in vitro HGP assay, L-DKO primary hepatocytes exhibited increases in HGP, glycogenolysis, and gluconeogenesis by 65%, 27%, and 71%, respectively ($P < 0.05$). Moreover, E_2 inhibited HGP and gluconeogenesis by 17% and 20%, respectively, in L-DKO cells (Fig. 5*F*). These results suggested that ovarian hormone protects against hyperglycemia induced by loss of hepatic insulin function and that E_2-mediated suppression of hepatic gluconeogenesis requires the activation of PI3K-Akt-Foxo1 signaling but in an Irs1- and Irs2-independent manner.

Activation of ERα Is Necessary and Sufficient to Activate Akt-Foxo1 Signaling and Suppress Gluconeogenesis in Hepatocytes

ERα is the major form of ERs in the liver (28). We next investigated whether ERα modulates E_2 effect on suppression of HGP in hepatocytes using its specific agonist and antagonist. ERα-selective antagonist methylpiperidinopyrazole (MPP) completely blocked E_2-reduced HGP, gluconeogenesis (Fig. 6*A*), and expression of *G6pc* and *Pck1* (Fig. 6*B*), as well as phosphorylation of Akt and Foxo1 (Fig. 6*C*). Moreover, MPP alone reduced basal phosphorylation of Akt and Foxo1 by 30% and 55%, respectively ($P < 0.05$) (Fig. 6*C*); this resulted in increases in *G6pc* and *Pck1* levels by 68% and 25% ($P < 0.05$) (Fig. 6*B*). ERα agonist propylpyrazoletriol (PPT) showed an effect similar to that of E_2 to reduce HGP and gluconeogenesis in both control (Fig. 6*A*) and L-DKO (Fig. 5*E*) hepatocytes. Meanwhile, PPT enhanced phosphorylation levels of Akt and Foxo1 by 2.7-fold and 1.8-fold, respectively ($P < 0.05$) and suppressed expression of *G6pc* by 30% in control hepatocytes ($P < 0.05$) (Fig. 6*B* and *C*).

We finally determined whether overexpression of ERα enhances insulin- or E_2-induced Akt and Foxo1 phosphorylation in HepG2 cells, human hepatoma cells. Insulin and E_2 increased Akt phosphorylation by 10-fold and 5.4-fold, respectively, and induced Foxo1 phosphorylation by 2.9-fold and 2.8-fold ($P < 0.05$). Overexpression of ERα enhanced basal phosphorylation of Akt and Foxo1 by 3.4-fold and 2.0-fold. Strikingly, ERα overexpression further enhanced insulin- and E_2-stimulated Akt phosphorylation by 67% and 42% and Foxo1 phosphorylation by 1.3- and 1.2-fold ($P < 0.05$) (Fig. 6*D*).

Taken together, these results indicate that ERα is necessary and sufficient for E_2 to activate Akt-Foxo1 signaling and suppress gluconeogenesis.

DISCUSSION

Estrogen has been shown to be involved in both central and peripheral regulation in glucose homeostasis (29,30). Estrogen deficiency or impaired estrogen signaling is associated with insulin resistance and dysregulation of metabolic homeostasis, thus contributing to the development of T2D and obesity in both human and animal models (31–33). However, the contribution of tissue-specific action of estrogen to metabolic changes and underlying mechanisms have yet to be elucidated. In this study, we have established that activation of ERα-Akt-Foxo1 signaling is an important mechanism for estrogen in the liver in maintaining glucose homeostasis.

In general, glucose homeostasis is maintained by glucose uptake in muscle and adipose tissue and endogenous glucose production in the liver. Previous studies reported that estrogen lowers glucose level, which is associated with enhanced glucose uptake in muscle through activation of Akt and induction of GLUT4 expression (10,34). However, blockage of E_2 signaling in global or liver ERα knockout mice causes hepatic insulin resistance and enhanced HGP, resulting in hyperglycemia (14,35). In this study, E_2 improved glucose tolerance in both sexes; however, such an effect was blocked upon deletion of hepatic Foxo1. Our results supported that improvement of glucose homeostasis by E_2 is regulated by hepatic Foxo1-mediated gluconeogenesis rather than by promoting muscle glucose uptake (12). In liver, Foxo1 promotes gluconeogenesis through activating transcription of *G6pc* and *Pck1* by directly binding to insulin response element CAAAACAA on their promoter regions (26,36). Liver-specific ablation of Foxo1 impairs gluconeogenesis and reduces fasting glucose level (37). Deletion of hepatic Foxo1 protects diabetic mice, such as L-DKO mice or *db/db* mice, from hepatic insulin resistance (23,27). In this study, suppression of gluconeogenesis by E_2 was abolished upon deletion of Foxo1; this supported

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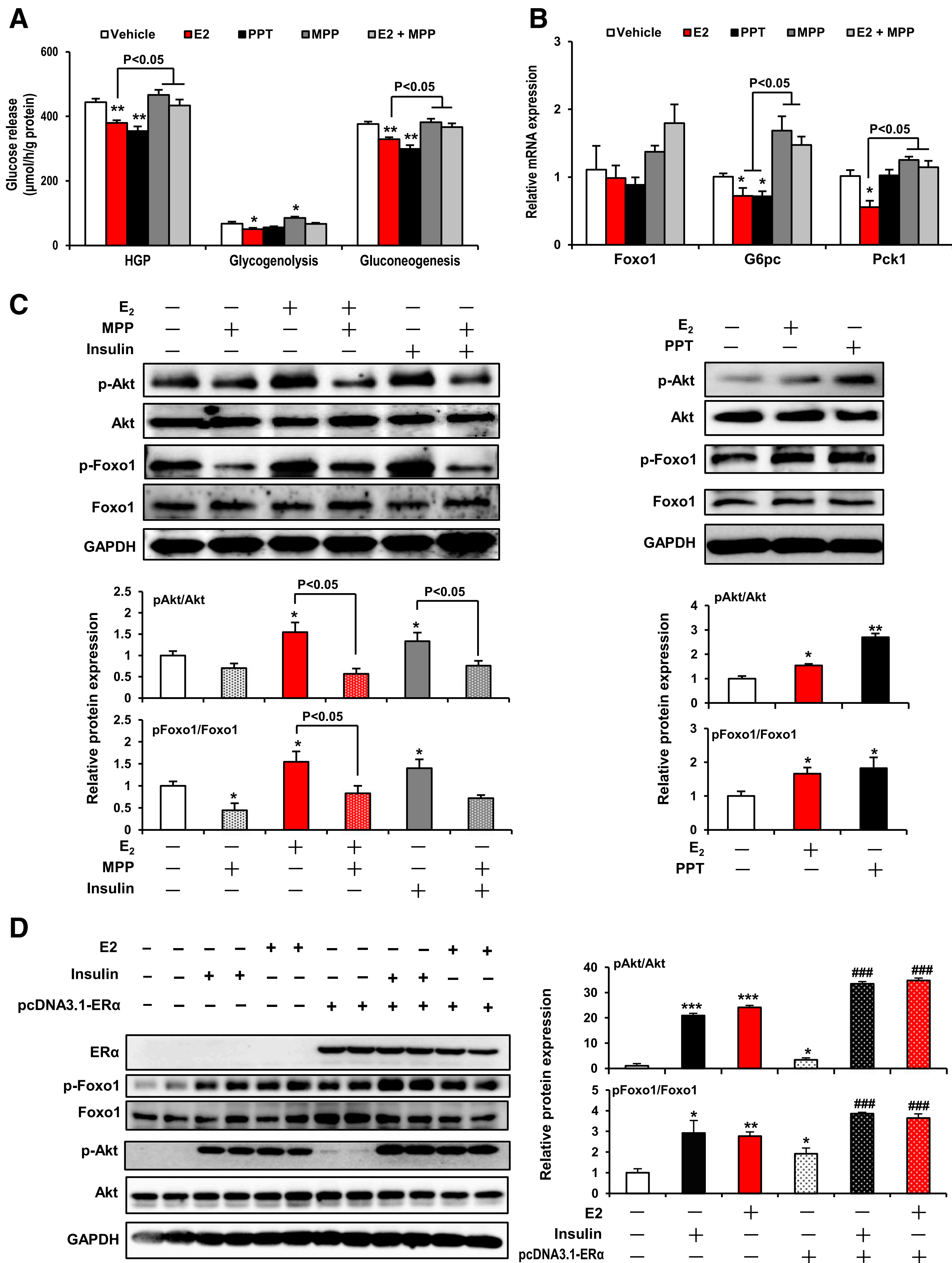

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Figure 6—ERα is required and sufficient to activate Akt-Foxo1 signaling and suppress gluconeogenesis in primary hepatocytes. *A*: Glucose production in primary hepatocytes from control mice in the presence (HGP) or absence (glycogenolysis) of pyruvate and lactate. Cells were treated with 1 μmol/L ERα antagonist MPP prior to stimulation of 0.1 μmol/L E_2 or 0.1 μmol/L ERα agonist PPT. *B*: Relative mRNA levels of

a notion that hepatic Foxo1 is required for E_2 action in the modulation of gluconeogenesis.

This study illustrated that E_2 inhibits Foxo1 transactivation via the interaction with ERα and activation of PI3K-Akt signaling, thus serving as a molecular basis for E_2 action on suppression of hepatic gluconeogenesis. ERα is involved in glucose homeostasis, and impaired ERα function is associated with insulin resistance, T2D, and metabolic syndrome in both human and animal models (14,15,35). Our results demonstrated that activation of ERα is required for E_2 to suppress HGP and that overexpression of ERα enhances insulin- and E_2-induced phosphorylation of Foxo1. Ambiguity about how ERα modulates insulin signaling and glucose metabolism still remains. A recent study indicated that an estrogen response element half-site (AGGTCA) presents in promoter regions of *G6pc* and *Pck1* (38). Recruitment of ERα to those promoters upon E_2 activation inhibits transcriptions of *G6pc* and *Pck1* (35), but Yasrebi et al. (39) reported that E_2 still decreased fasting glucose and improved glucose tolerance in the ERα knock-in mice, in which the knock-in mutation blocks the functional estrogen response element binding activity for DNA, ruling out this genomic signaling of ERα. An E_2-dependent binding of ERα with Foxo1 was reported to suppress Foxo1 transactivation in the MCF7 cell line (40). Inhibition of Akt signaling by inhibitors or by S253A mutant of the Akt phosphorylation site in Foxo1 totally blocked effects of estrogen on HGP; this suggested the importance of indirect E_2 action on Foxo1 Ser^{253} phosphorylation via ERα-PI3K-Akt rather than a direct interaction between ERα and Foxo1. Direct interactions of ERα with several proteins in the insulin signaling cascade have been reported. ERα binds to Irs1 and Irs2 and promotes stability of the ERα/Irs complex in breast cancer lines (41,42); it also binds to the p85α catalytic subunit of PI3K in human embryonic kidney (HEK)293T, MCF7, and aortic endothelial cells (43,44). A direct interaction between p85α and ERα in proopiomelanocortin (POMC) progenitor neurons has been reported, and deletion of PI3K in POMC neurons attenuates E_2-reduced glucose level in OVX females (45). However, the question of whether these protein interactions contribute to hepatic glucose metabolism remains. In this study, L-DKO intact females were protected from hyperglycemia and deletion of Irs1 and Irs2 did not disrupt E_2-suppressed fasting glucose level in vivo or hepatic gluconeogenesis in vitro, whereas PI3K inhibitor totally blocked the E_2 action; this suggested that the interaction of ERα with PI3K may contribute to activation of Akt-Foxo1 and regulation of glucose homeostasis independent of Irs1 and Irs2. The phosphorylation sites of Foxo1 by Akt are also identified in other Foxo members, including Foxo3 and Foxo4. Our previous study indicated that Foxo1, rather than Foxo3 and -4, significantly influences glucose homeostasis (21). Even if E_2 modifies the activity of other Foxo isoforms via Akt, the contribution of Foxo3 and Foxo4 by E_2 to glucose metabolism is limited.

E_2 exhibits a Foxo1-independent mechanism to reduce glucose level only in females. OVX decreased hepatic glycogen (46), which was restored by E_2, as previously reported (11,12,47,48). E_2 increased glycogen independent of hepatic Foxo1, which may account for the reduced glucose level in L-F1KO females. Hepatic glycogen is regulated by glycogen synthase kinase 3α (GSK3α), which phosphorylates glycogen synthase and inhibits glycogen synthesis (49). GSK3α is inactivated by Akt-mediated phosphorylation, and loss of function of GSK3α is associated with increased hepatic glycogen and lowered glucose levels (50). Thus, we speculate that activation of Akt and inhibition of GSK3α to promote glycogen synthesis by E_2 may be involved in a Foxo1-independent mechanism for E_2 to reduce glucose level in females. In fact, a study reported that hepatic Foxo1 ablation is associated with an increase in liver glycogen and inhibition of glycogenolysis in hepatocytes (37); another study indicated that Foxo1 has limited effects on hepatic glycogen (20). Nevertheless, we found that deficiency of hepatic Foxo1 increased liver glycogen only in OVX females, which is masked by E_2 implant. That deletion of hepatic Foxo1 increased liver glycogen might be the result of inhibition of gluconeogenesis and accumulation of glucose-6-phosphate, a substrate for glycogen synthesis.

Sex differences exist in liver glycogen levels and responses to E_2. Male mice exhibited an 85% reduction in liver glycogen compared with intact females, and E_2-increased hepatic glycogen levels as observed in OVX females disappeared in male mice. Several studies have indicated that long-term exercise training increases glycogen storage in both muscle and liver with enhanced glycogen synthase activity (51). Female mice naturally exhibited a higher level of physical activity than male mice (52); we suspect that such a higher physical activity may contribute to a greater capacity to store glycogen. This also implies a sexual dimorphism in the contribution of glycogen metabolism to glucose homeostasis.

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gluconeogenic genes in primary hepatocytes from control mice upon stimulation of 0.1 μmol/L E_2, 1 μmol/L MPP, or 0.1 μmol/L PPT. *C*: Western blots and corresponding quantification of insulin-signaling protein in hepatocytes from control mice upon stimulation of 0.1 μmol/L E_2, 1 μmol/L MPP, or 0.1 μmol/L PPT. *D*: Western blots and corresponding quantification of Foxo1 protein in HepG2 cells upon overexpression of ERα and stimulation of E_2 or insulin. HepG2 cells were transfected with 10 μg plasmid DNA expressing green fluorescent protein (GFP) or ERα for 30 h, followed by 6 h starvation prior to 0.1 μmol/L E_2 or 0.1 μmol/L insulin stimulation for 1 h. All data are expressed as mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. control + vehicle. ###*P* < 0.001 vs. control + ERα overexpression. *n* = 3 of each group. p- or p, phosphorylated.

E_2 suppressed body weight in OVX females independent of hepatic Foxo1 but reduced leptin level dependent on Foxo1, whereas E_2 was less effective in reduction of body weight and leptin in males. Our previous studies showed that estrogen increases energy expenditure and decreases body weight through binding to its receptors in the central nervous system (CNS) (53). In the OVX female mice, obesity developed and E_2 administration reduced the body weight and serum leptin level, suggesting E_2 increases the leptin sensitivity, which is likely via increasing expression of Irs2, a modulator of leptin action in the CNS (54). Leptin level is highly associated with the amount of fat mass. E_2-decreased fat mass accumulation through CNS may explain the decreased leptin level. However, E_2 administration reduced the body weight in L-F1KO mice; the effect can be independent of leptin level but dependent on leptin sensitivity with an increase of Irs2 expression and energy expenditure (54). The sex differences may result from differences in expression of ER isoforms in various tissues (55), absorbance rates of exogenous E_2 and local E_2 concentrations, and capacity of E_2 to cross the blood-brain barrier to CNS.

Estrogen is critical to maintaining metabolic homeostasis in both sexes (31). In this study, E_2 implant improved insulin sensitivity and lowered fasting glucose in both sexes. In premenopausal women, E_2 is converted from androstenedione by aromatization in the ovaries and is released as circulating hormone acting on distant tissues. In men, E_2 is produced locally in extragonadal tissues by aromatization from testosterone (31,56). Patients lacking aromatase function and aromatase-deficient mice (ArKO mice), with endogenous estradiol synthesis diminished in both cases, exhibit impaired glucose homeostasis (3,57). Men lacking aromatase also exhibit hyperinsulinemia (58), and male ArKO mice develop insulin resistance (57); this suggests that E_2 signaling is similarly crucial in metabolic homeostasis in both sexes. Of note, L-F1KO males had a detectable serum E_2 level compared with the undetectable serum E_2 in control males. Given that deletion of hepatic Foxo1 increases cholesterol levels (59) and cholesterol is a precursor for steroid hormone biosynthesis, we speculate that hepatic Foxo1 may also be involved in estrogen biosynthesis indirectly through the regulation of cholesterol production in the liver.

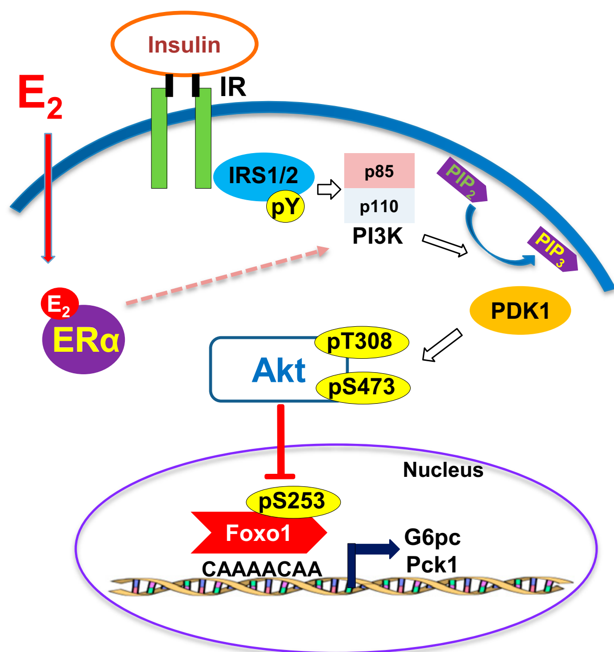

Figure 7—Schematic diagram represents the role of estrogen in the regulation of glucose metabolism. Estrogen suppresses hepatic gluconeogenesis and lowers blood glucose through interaction with ERα in a Foxo1-dependent manner. E_2 inhibits Foxo1 and its target *G6pc* expression indirectly, depending on the activation of PI3K-Akt signaling. IR, insulin receptor; p, phosphorylated; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-trisphosphate; PDK1, 3-phosphoinositide-dependent protein kinase 1; Y, tyrosin; T, threonine; S, serine.

In summary, our study demonstrated that E_2 improves insulin sensitivity and suppresses hepatic gluconeogenesis through inhibition of Foxo1 via activation of ERα-PI3K-Akt signaling (Fig. 7). PI3K is required for E_2 action, but comprehensive studies into the direct interaction of ERα with PI3K regulating hepatic glucose metabolism have not been conducted. Since estrogen promotes the development of the reproductive system, the metabolism and reproductive biology can be regulated by different sets of target genes. Therefore, the identification of tissue-specific actions of E_2 and direct targets of ERs will facilitate the development of novel selective ligands that prevent T2D, cardiovascular disease, and obesity without promoting abnormal sex characteristics or breast cancer.

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Author Contributions. H.Y., W.Y., and F.Z. designed and conducted experiments and performed data analyses. H.Y. and S.G. wrote the manuscript. X.L., Q.P., Z.S., and K.A. conducted experiments. G.H., A.N.-F., Y.T., R.M., W.L., Y.X., C.W., C.A., and Y.S. reviewed and edited the manuscript. S.G. supervised the project, conceived of the hypothesis, and designed experiments. S.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Danaei G, Finucane MM, Lu Y, et al.; Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Blood Glucose). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. Lancet 2011;378: 31–40

2. Salpeter SR, Walsh JM, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. Diabetes Obes Metab 2006;8: 538–554

3. Misso ML, Murata Y, Boon WC, Jones ME, Britt KL, Simpson ER. Cellular and molecular characterization of the adipose phenotype of the aromatase-deficient mouse. Endocrinology 2003;144:1474–1480

4. Louet J-F, LeMay C, Mauvais-Jarvis F. Antidiabetic actions of estrogen: insight from human and genetic mouse models. Curr Atheroscler Rep 2004;6: 180–185

5. Kim C, Kong S, Laughlin GA, et al.; Diabetes Prevention Program Research Group. Reductions in glucose among postmenopausal women who use and do not use estrogen therapy. Menopause 2013;20:393–400

6. Manson JE, Chlebowski RT, Stefanick ML, et al. The Women's Health Initiative Hormone Therapy Trials: Update and Overview of Health Outcomes During the Intervention and Post-Stopping Phases. JAMA 2013;310:1353–1368

7. Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Suissa S, Horwitz RI. A clinical trial of estrogen-replacement therapy after ischemic stroke. N Engl J Med 2001; 345:1243–1249

8. Stefanick ML, Anderson GL, Margolis KL, et al.; WHI Investigators. Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. JAMA 2006;295:1647–1657

9. Pilkis SJ, Granner DK. Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annu Rev Physiol 1992;54:885–909

10. Moreno M, Ordoñez P, Alonso A, Díaz F, Tolivia J, González C. Chronic 17beta-estradiol treatment improves skeletal muscle insulin signaling pathway components in insulin resistance associated with aging. Age (Dordr) 2010;32:1–13

11. Matute ML, Kalkhoff RK. Sex steroid influence on hepatic gluconeogenesis and glucogen formation. Endocrinology 1973;92:762–768

12. Sladek CD. The effects of human chorionic somatomammotropin and estradiol on gluconeogenesis and hepatic glycogen formation in the rat. Horm Metab Res 1975;7:50–54

13. Carter S, McKenzie S, Mourtzakis M, Mahoney DJ, Tarnopolsky MA. Short-term 17β-estradiol decreases glucose R(a) but not whole body metabolism during endurance exercise. J Appl Physiol (1985) 2001;90:139–146

14. Bryzgalova G, Gao H, Ahren B, et al. Evidence that oestrogen receptor-alpha plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver. Diabetologia 2006;49:588–597

15. Gao H, Fält S, Sandelin A, Gustafsson JA, Dahlman-Wright K. Genome-wide identification of estrogen receptor alpha-binding sites in mouse liver. Mol Endocrinol 2008;22:10–22

16. Schmoll D, Walker KS, Alessi DR, et al. Regulation of glucose-6-phosphatase gene expression by protein kinase Balpha and the forkhead transcription factor FKHR. Evidence for insulin response unit-dependent and -independent effects of insulin on promoter activity. J Biol Chem 2000;275:36324–36333

17. Ayala JE, Streeper RS, Desgrosellier JS, et al. Conservation of an insulin response unit between mouse and human glucose-6-phosphatase catalytic subunit gene promoters: transcription factor FKHR binds the insulin response sequence. Diabetes 1999;48:1885–1889

18. Zhao X, Gan L, Pan H, et al. Multiple elements regulate nuclear/cytoplasmic shuttling of FOXO1: characterization of phosphorylation- and 14-3-3-dependent and -independent mechanisms. Biochem J 2004;378:839–849

19. Guo S. Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models into disease mechanisms. J Endocrinol 2014;220:T1–T23

20. O-Sullivan I, Zhang W, Wasserman DH, et al. FoxO1 integrates direct and indirect effects of insulin on hepatic glucose production and glucose utilization [published correction appears in Nat Commun 2015;6:7861]. Nat Commun 2015; 6:7079

21. Zhang K, Li L, Qi Y, et al. Hepatic suppression of Foxo1 and Foxo3 causes hypoglycemia and hyperlipidemia in mice. Endocrinology 2012;153: 631–646

22. Michael MD, Kulkarni RN, Postic C, et al. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 2000;6:87–97

23. Dong XC, Copps KD, Guo S, et al. Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. Cell Metab 2008;8:65–76

24. Lo S, Russell JC, Taylor AW. Determination of glycogen in small tissue samples. J Appl Physiol 1970;28:234–236

25. Pajvani UB, Shawber CJ, Samuel VT, et al. Inhibition of Notch signaling ameliorates insulin resistance in a FoxO1-dependent manner. Nat Med 2011;17: 961–967

26. Guo S, Rena G, Cichy S, He X, Cohen P, Unterman T. Phosphorylation of serine 256 by protein kinase B disrupts transactivation by FKHR and mediates effects of insulin on insulin-like growth factor-binding protein-1 promoter activity through a conserved insulin response sequence. J Biol Chem 1999;274:17184–17192

27. Guo S, Copps KD, Dong X, et al. The Irs1 branch of the insulin signaling cascade plays a dominant role in hepatic nutrient homeostasis. Mol Cell Biol 2009; 29:5070–5083

28. Kuiper GG, Carlsson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 1997;138:863–870

29. Barros RP, Gustafsson JA. Estrogen receptors and the metabolic network. Cell Metab 2011;14:289–299

30. Faulds MH, Zhao C, Dahlman-Wright K, Gustafsson JA. The diversity of sex steroid action: regulation of metabolism by estrogen signaling. J Endocrinol 2012; 212:3–12

31. Mauvais-Jarvis F. Estrogen and androgen receptors: regulators of fuel homeostasis and emerging targets for diabetes and obesity. Trends Endocrinol Metab 2011;22:24–33

32. Hevener AL, Clegg DJ, Mauvais-Jarvis F. Impaired estrogen receptor action in the pathogenesis of the metabolic syndrome. Mol Cell Endocrinol 2015;418:306–321

33. Zhu L, Brown WC, Cai Q, et al. Estrogen treatment after ovariectomy protects against fatty liver and may improve pathway-selective insulin resistance. Diabetes 2013;62:424–434

34. Gorres BK, Bomhoff GL, Morris JK, Geiger PC. In vivo stimulation of oestrogen receptor α increases insulin-stimulated skeletal muscle glucose uptake. J Physiol 2011;589:2041–2054

35. Qiu S, Vazquez JT, Boulger E, et al. Hepatic estrogen receptor α is critical for regulation of gluconeogenesis and lipid metabolism in males. Sci Rep 2017;7:1661

36. Onuma H, Vander Kooi BT, Boustead JN, Oeser JK, O'Brien RM. Correlation between FOXO1a (FKHR) and FOXO3a (FKHRL1) binding and the inhibition of basal glucose-6-phosphatase catalytic subunit gene transcription by insulin. Mol Endocrinol 2006;20:2831–2847

37. Matsumoto M, Pocai A, Rossetti L, Depinho RA, Accili D. Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor Foxo1 in liver. Cell Metab 2007;6:208–216

38. Chen H, Hu B, Gacad MA, Adams JS. Cloning and expression of a novel dominant-negative-acting estrogen response element-binding protein in the heterogeneous nuclear ribonucleoprotein family. J Biol Chem 1998;273:31352–31357

39. Yasrebi A, Rivera JA, Krumm EA, Yang JA, Roepke TA. Activation of estrogen response element-independent ERα signaling protects female mice from diet-induced obesity. Endocrinology 2017;158:319–334

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40. Schuur ER, Loktev AV, Sharma M, Sun Z, Roth RA, Weigel RJ. Ligand-dependent interaction of estrogen receptor-alpha with members of the forkhead transcription factor family. J Biol Chem 2001;276:33554–33560
41. Sisci D, Morelli C, Cascio S, et al. The estrogen receptor alpha:insulin receptor substrate 1 complex in breast cancer: structure-function relationships. Ann Oncol 2007;18(Suppl. 6):vi81–vi85
42. Morelli C, Garofalo C, Bartucci M, Surmacz E. Estrogen receptor-alpha regulates the degradation of insulin receptor substrates 1 and 2 in breast cancer cells. Oncogene 2003;22:4007–4016
43. Sun M, Paciga JE, Feldman RI, et al. Phosphatidylinositol-3-OH Kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor alpha (ERalpha) via interaction between ERalpha and PI3K. Cancer Res 2001;61:5985–5991
44. Simoncini T, Hafezi-Moghadam A, Brazil DP, Ley K, Chin WW, Liao JK. Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature 2000;407:538–541
45. Zhu L, Xu P, Cao X, et al. The ERα-PI3K cascade in proopiomelanocortin progenitor neurons regulates feeding and glucose balance in female mice. Endocrinology 2015;156:4474–4491
46. Kumagai S, Holmäng A, Björntorp P. The effects of oestrogen and progesterone on insulin sensitivity in female rats. Acta Physiol Scand 1993;149:91–97
47. Ahmed-Sorour H, Bailey CJ. Role of ovarian hormones in the long-term control of glucose homeostasis, glycogen formation and gluconeogenesis. Ann Nutr Metab 1981;25:208–212
48. Bitman J, Cecil HC, Mench ML, Wrenn TR. Kinetics of in vivo glycogen synthesis in the estrogen-stimulated rat uterus. Endocrinology 1965;76:63–69
49. Roach PJ, Depaoli-Roach AA, Hurley TD, Tagliabracci VS. Glycogen and its metabolism: some new developments and old themes. Biochem J 2012;441:763–787
50. MacAulay K, Doble BW, Patel S, et al. Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. Cell Metab 2007;6:329–337
51. Murakami T, Shimomura Y, Fujitsuka N, Sokabe M, Okamura K, Sakamoto S. Enlargement glycogen store in rat liver and muscle by fructose-diet intake and exercise training. J Appl Physiol (1985) 1997;82:772–775
52. Lightfoot JT. Sex hormones' regulation of rodent physical activity: a review. Int J Biol Sci 2008;4:126–132
53. Xu Y, Nedungadi TP, Zhu L, et al. Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. Cell Metab 2011;14:453–465
54. Sadagurski M, Leshan RL, Patterson C, et al. IRS2 signaling in LepR-b neurons suppresses FoxO1 to control energy balance independently of leptin action. Cell Metab 2012;15:703–712
55. Matic M, Bryzgalova G, Gao H, et al. Estrogen signalling and the metabolic syndrome: targeting the hepatic estrogen receptor alpha action. PLoS One 2013;8:e57458
56. Simpson ER. Sources of estrogen and their importance. J Steroid Biochem Mol Biol 2003;86:225–230
57. Jones ME, Thorburn AW, Britt KL, et al. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci U S A 2000;97:12735–12740
58. Jones ME, Boon WC, Proietto J, Simpson ER. Of mice and men: the evolving phenotype of aromatase deficiency. Trends Endocrinol Metab 2006;17:55–64
59. Haeusler RA, Pratt-Hyatt M, Welch CL, Klaassen CD, Accili D. Impaired generation of 12-hydroxylated bile acids links hepatic insulin signaling with dyslipidemia. Cell Metab 2012;15:65–74

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