Males Require Estrogen Signaling Too: Sexual Dimorphism in the Regulation of Glucose Homeostasis by Nuclear ERα

Emilyn Uy Alejandro

Diabetes 2019;68:471–473 | https://doi.org/10.2337/dbi18-0046

Females are more protected against insulin resistance and cardiovascular disease compared with males of the same age or BMI, and this relative protection is diminished in postmenopausal women, attributable to a loss in estrogen signaling (1–4). Evidence from genetic animal models further points to the beneficial effects of estrogen signaling via estrogen receptor-α (ERα) in regard to glucose homeostasis in health and metabolic diseases (5). Mice harboring whole-body deletion of ERα (ERαKO) reveal that estrogen signaling through this receptor regulates glucose homeostasis in part by modulating hepatic insulin sensitivity (6) and glucose uptake in the skeletal muscle and adipose tissue (7). Moreover, ERα signaling has been demonstrated to promote pancreatic insulin-producing β-cell function, survival, and proliferation, as well as protection from development of diabetes in mice of both sexes (8–10). Thus, estrogen is generally thought to positively regulate glucose homeostasis primarily through ERα, which is expressed in both male and female tissues, but the respective importance of nuclear and membrane ERα pools in the regulation of glucose homeostasis is not clear.

ERα is predominantly characterized as a nuclear receptor and thus noted for exclusively regulating transcription of target genes. However, extranuclear ERα action or membrane compartment–initiated estrogen signaling is now widely accepted to activate different pathways occurring in the cytoplasm and nucleus. For example, membrane localization of ERα (which accounts for ∼5–10% of the ERα pool depending on cell type) facilitates membrane-initiated signaling events important for reproduction and vascular physiology (11) as well as β-cell function and survival (12). Although it is clear that ERα positively regulates glucose homeostasis, the distinct and overlapping contributions of the extranuclear and nuclear pool of ERα remain unknown. Therefore, mouse models specifically engineered to have only nuclear or membrane ERα action are necessary to delineate specific ERα functions related to its subcellular localization, which will give us much needed insight into the mechanisms underlying the beneficial effects of estrogen on insulin resistance and diabetes observed in both clinical and animal studies.

In this issue of *Diabetes*, Allard et al. (13) aimed to interrogate the relative contribution of nuclear and membrane ERα in glucose homeostasis. This research endeavor is important because their findings refine our knowledge of the actions of ERα and may impact our ability to target specific action of ERα in critical tissues, a potential avenue for the development of sex-based therapy for diabetes. First, they show that female mice expressing exclusively nuclear ERα (NOER) or membrane ERα (MOER) developed glucose intolerance by 6 months of age. However, only male MOER mice, expressing membrane but not nuclear ERα, demonstrated hyperglycemia associated with glucose intolerance (assessed via intraperitoneal injection [i.p.] glucose tolerance test). It is important to note that both female and male mice with global ERα deficiency (ERαKO) are glucose intolerant and insulin resistant based on elevated insulin levels (14) or reduced glucose infusion rate via euglycemic-hyperinsulinemic clamp (15,16). Allard et al. also reported that female MOER mice, like female ERαKO mice, exhibited resistance to the hypoglycemic effect of insulin (via i.p. insulin tolerance test [ITT]), whereas female NOER mice exhibited normal insulin sensitivity (via ITT). By contrast, all male mice (MOER, NOER, and ERαKO) exhibited comparable and relatively normal responses to an ITT (13). The authors further supported their conclusion on insulin resistance by demonstrating that both female MOER mice and female NOER mice show a decreased glucose infusion rate in a hyperinsulinemic-euglycemic clamp study, a gold-standard

Department of Integrative Biology and Physiology, University of Minnesota Medical School, Minneapolis, MN

Corresponding author: Emilyn Uy Alejandro, ealejand@umn.edu

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/dbi18-0046/-/DC1.

See accompanying article, p. 490.

technique to assess insulin sensitivity. Others have also reported that female ERαKO mice displayed insulin resistance via assessment of glucose infusion rate (15). The metabolic phenotypes of mice are summarized in Supplementary Table 1. The authors conclude that loss of nuclear ERα, and to a lesser extent membrane ERα, impairs glucose homeostasis in mice of both sexes.

Impaired glucose intolerance in male MOER and ERαKO mice can be explained in part by impairment in pancreatic β-cell insulin secretion, as these mice display normal insulin sensitivity. Allard et al. (13) show that when male MOER mice were challenged for glucose-stimulated insulin secretion (GSIS) in vivo, they demonstrated a blunted response only during the first 5 min post–i.p. glucose injection (first phase of insulin secretion) but increased insulin levels after 30 min postglucose (second phase). They go on to demonstrate that the first-phase insulin secretion defect in male MOER mice is not observed in static incubation of islets in vitro; the authors concluded that the in vivo defect in first-phase insulin secretion in GSIS is independent of the loss of nuclear ERα in β-cells and secondary to the loss of ERα in extraislet tissues, possibly impairing islet function via a neural factor yet to be defined. Allard et al. (13) did not elaborate on possible explanations for the increased insulin levels observed during the second phase of insulin secretion in male MOER mice. Next, the authors show that female MOER mice demonstrated normal insulin secretion during the first phase of GSIS in vivo. Like male MOER mice (with normal insulin sensitivity), female MOER mice (insulin resistant) demonstrated hyperinsulinemia in the second phase of insulin secretion. Female MOER fed and fasted insulin levels, as well as basal plasma insulin prior to hyperinsulinemic-euglycemic clamp, were significantly elevated, suggesting β-cell hypersecretion. As previously mentioned, female MOER mice demonstrated significant insulin resistance. Thus, the β-cells appear to be responding to insulin resistance by increasing insulin levels. Total pancreatic insulin content in male and female MOER mice was normal, suggesting a lack of change in β-cell mass. The estimated β-cell mass between male MOER and control mice was also comparable. This is a surprise finding given the reported effects of ERα on insulin gene expression (17,18) and on β-cell survival and proliferation (9,19). It is interesting to also point out that female NOER showed normal glucose tolerance despite demonstrating insulin resistance. In this model, β-cell hypersecretion of insulin appears also to compensate for insulin resistance in NOER mice. Future studies should be directed toward identifying mechanisms by which ERα modulates temporal regulation of insulin secretion. It would be ideal in the future to use an islet perfusion technique to assess temporal dynamics (first and second phases of insulin secretion) compared to a static incubation test.

So what are the central mechanisms of impaired glucose homeostasis in the MOER mice? The central nervous system (CNS) integrates information regarding peripheral nutrient and hormonal changes and processes this information to regulate energy homeostasis. Recent findings indicate that some of the neural circuits and mechanisms underlying energy balance are also essential for the regulation of glucose homeostasis. Allard et al. (13) propose that ERα activity in the CNS stimulates pancreatic insulin secretion in males but promotes liver insulin sensitivity in females. Female MOER mice exhibited central insulin resistance, as determined by the failure of a central insulin infusion to activate Akt in the hippocampus or IL-6–STAT3 in the liver, leading to unsuppressed hepatic glucose production. Female ERαKO mice demonstrated hepatic insulin resistance just like female MOER and to a lesser degree female NOER (20). Hepatocyte-specific ablation of ERα (LERKO mice) has also been shown to induce insulin resistance in females (20,21). Therefore, ERα in the liver is important to whole-body and liver insulin sensitivity in female mice. While agouti-related protein (AgRP) neurons in the hypothalamus are thought to regulate hepatic glucose production (22), ERα has been shown to be completely excluded from AgRP and neuropeptide Y neurons (23). ERα is expressed in mouse proopiomelanocortin in hypothalamic (POMC) neurons (24), where its deletion

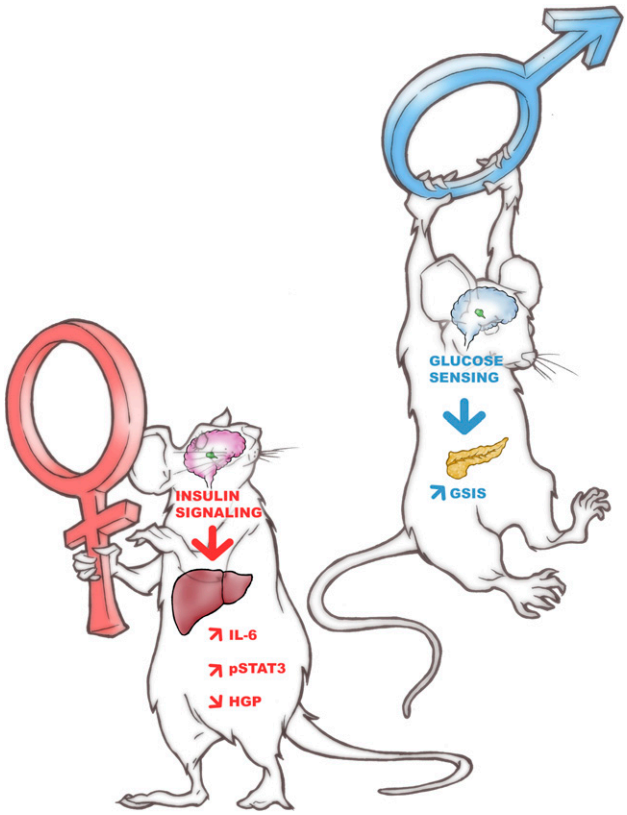

Figure 1—Sexual dimorphism in glucose homeostasis regulation by nuclear ERα. In female mice, nuclear ERα signaling in the brain promotes suppression of hepatic glucose production (HGP) via a brain-liver IL-6–STAT3 pathway. In male mice, ERα in the brain regulates glucose-stimulated first-phase insulin secretion. pSTAT3, phosphorylated STAT3.

leads to insulin resistance just like in female MOER mice. Therefore, it is likely that loss of ERα in POMC neurons can alter neuronal excitability of AgRP neurons, leading to changes in hepatic glucose production. Nevertheless, it is worth noting that the phenotype of the POMC ERαKO mice is not as striking as the MOER mice, suggesting that ERα action in POMC neurons is not sufficient to explain the full phenotype of the MOER mice. Thus, additional studies are needed to substantiate the role of ERα POMC neurons in the context of hepatic glucose production and insulin secretion.

In summary, Allard et al. (13) bring an important and timely first insight on the distinct role of nuclear and membrane ERα on glucose homeostasis (Fig. 1). Overall, the study brings a compelling finding that global deletion of nuclear ERα, and to a lesser extent membrane ERα, alters the central control of hepatic glucose production in female mice. However, in male mice, lack of nuclear ERα predominantly impairs the central regulation of insulin secretion. One of the key unanswered questions emerging from this study is which neuronal population is mediating these sexually dimorphic effects of ERα on glucose homeostasis? It will also be critical to show direct CNS manipulation of MOER and NOER to confirm that this is the central mechanism of ERα action in this context. Moreover, it will be important in the future to assess the phenotypes of mice with temporal and conditional tissue deletion of MOER and NOER to demonstrate direct causal effects. Subsequently, we can pinpoint viable tissue-specific targets for sex-based therapies for diabetes.

Acknowledgments. The author thanks Ronald Mark Barcenilla Ygona (Minneapolis, MN) for providing the Fig. 1 illustration.

Funding. Work in E.U.A.'s laboratory was supported by the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases (K01DK103823, R21DK112144, R03DK114465, and R01DK115720).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

References

1. Go AS, Mozaffarian D, Roger VL, et al.; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. Circulation 2013;127:e6–e245

2. Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR. Menopause and risk factors for coronary heart disease. N Engl J Med 1989;321:641–646

3. Mauvais-Jarvis F, Manson JE, Stevenson JC, Fonseca VA. Menopausal hormone therapy and type 2 diabetes prevention: evidence, mechanisms, and clinical implications. Endocr Rev 2017;38:173–188

4. Wedisinghe L, Perera M. Diabetes and the menopause. Maturitas 2009;63:200–203

5. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. Endocr Rev 2013;34:309–338

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

7. Barros RP, Gabbi C, Morani A, Warner M, Gustafsson JA. Participation of ERα and ERβ in glucose homeostasis in skeletal muscle and white adipose tissue. Am J Physiol Endocrinol Metab 2009;297:E124–E133

8. Kilic G, Alvarez-Mercado AI, Zarrouki B, et al. The islet estrogen receptor-α is induced by hyperglycemia and protects against oxidative stress-induced insulin-deficient diabetes. PLoS One 2014;9:e87941

9. Le May C, Chu K, Hu M, et al. Estrogens protect pancreatic β-cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. Proc Natl Acad Sci U S A 2006;103:9232–9237

10. Mauvais-Jarvis F, Le May C, Tiano JP, Liu S, Kilic-Berkmen G, Kim JH. The role of estrogens in pancreatic islet physiopathology. Adv Exp Med Biol 2017;1043:385–399

11. Adlanmerini M, Solinhac R, Abot A, et al. Mutation of the palmitoylation site of estrogen receptor α in vivo reveals tissue-specific roles for membrane versus nuclear actions. Proc Natl Acad Sci U S A 2014;111:E283–E290

12. Liu S, Le May C, Wong WP, et al. Importance of extranuclear estrogen receptor-α and membrane G protein–coupled estrogen receptor in pancreatic islet survival. Diabetes 2009;58:2292–2302

13. Allard C, Morford JJ, Xu B, et al. Loss of nuclear and membrane estrogen receptor-α differentially impairs insulin secretion and action in male and female mice. Diabetes 2019:68:490–501

14. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-α knockout mice. Proc Natl Acad Sci U S A 2000;97:12729–12734

15. Manrique C, Lastra G, Habibi J, Mugerfeld I, Garro M, Sowers JR. Loss of estrogen receptor α signaling leads to insulin resistance and obesity in young and adult female mice. Cardiorenal Med 2012;2:200–210

16. Riant E, Waget A, Cogo H, Arnal JF, Burcelin R, Gourdy P. Estrogens protect against high-fat diet-induced insulin resistance and glucose intolerance in mice. Endocrinology 2009;150:2109–2117

17. Alonso-Magdalena P, Ropero AB, Carrera MP, et al. Pancreatic insulin content regulation by the estrogen receptor ERα. PLoS One 2008;3:e2069

18. Wong WP, Tiano JP, Liu S, et al. Extranuclear estrogen receptor-α stimulates NeuroD1 binding to the insulin promoter and favors insulin synthesis. Proc Natl Acad Sci U S A 2010;107:13057–13062

19. Yuchi Y, Cai Y, Legein B, et al. Estrogen receptor α regulates β-cell formation during pancreas development and following injury. Diabetes 2015;64:3218–3228

20. 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

21. Zhu L, Shi J, Luu TN, et al. Hepatocyte estrogen receptor alpha mediates estrogen action to promote reverse cholesterol transport during Western-type diet feeding. Mol Metab 2018;8:106–116

22. Könner AC, Janoschek R, Plum L, et al. Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. Cell Metab 2007;5:438–449

23. Olofsson LE, Pierce AA, Xu AW. Functional requirement of AgRP and NPY neurons in ovarian cycle-dependent regulation of food intake. Proc Natl Acad Sci U S A 2009;106:15932–15937

24. de Souza FS, Nasif S, López-Leal R, Levi DH, Low MJ, Rubinsten M. The estrogen receptor α colocalizes with proopiomelanocortin in hypothalamic neurons and binds to a conserved motif present in the neuron-specific enhancer nPE2. Eur J Pharmacol 2011;660:181–187