Minireview: The Year in Review of Estrogen Regulation of Metabolism

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Gonadal steroids are critical regulators of physiology, yet we approach physiology and science with the simplest perspective/model, the male one. Female models of whole animal physiology are complex to study and, therefore, are often not used in research. Estrogens are one of the sex hormones that we know are important for both men and women. Estrogens regulate key features of metabolism such as food intake, body weight, glucose homeostasis/insulin sensitivity, body fat distribution, lipolysis/lipogenesis, inflammation, locomotor activity, energy expenditure, reproduction, and cognition. Furthermore, estrogens have multiple sites of action including some unexpected ones, which was demonstrated elegantly through a series of papers this year. **(*Molecular Endocrinology* 26: 1957–1960, 2012)**

Two basic estrogen receptors (ERs) are important in overall physiology, ERα and -β. ERα (*i.e.* NR3A1) is particularly relevant for several reasons: Human inactivating mutations of ERα recapitulate aspects of the metabolic syndrome. Diminished ERα activity is associated with obesity in both women and men. Furthermore, impaired ERα action is a contributing factor in the pathobiology of the metabolic syndrome. Much of our understanding of the overall metabolic role of ERα were initially uncovered in estrogen receptor α-null mouse ($ER\alpha^{-/-}$). Specifically, mice with a homozygous *Esr1*-null mutations show glucose intolerance, inflammation, insulin resistance, and obesity and, in essence, recapitulate the metabolic syndrome.

Research reviewed in the first highlighted paper (1) focused on augmenting ERα in the brain, specifically the central nervous system, and evaluating outcomes between males and females. These experiments were motivated by important observations from Elmquist *et al.* (2) and several others, that critical regulators of metabolism have receptors in the central nervous system that mediate overall energy expenditure, food intake, and glucose homeostasis. Our research questions were whether estrogens specifically augment the efficacy of these factors, and/or whether estrogens bind to specific regulators in the central nervous system to modulate energy homeostasis and the like. Identification of the contribution of brain-specific ERs with respect to the overall metabolic phenotype of those animals was the goal. ERα floxed mice were bred to Nestin-CRE mice, which allowed us to excise ERα from the brain. Brain regions such as the arcuate nucleus and the periventricular nucleus, as well as the brainstem, are important for modulating or mediating overall energy homeostasis, and ERs are located in these regions. Both males and females had a significant increase in total body weight, which provided evidence that the brain is a critical site of estrogenic action. Importantly, others have also demonstrated that the periphery is also a site of estrogenic action that regulates metabolism and this will be discussed later. Mice lacking ERα from the central nervous system became obese through increased caloric intake and reduced energy expenditure. Additionally, there was a significant reduction in overall activity and a significant increase in plasma 17β-estradiol levels. These data further emphasized that the brain is a critical site for negative feedback regulation of the HPG axis with respect to FSH and GnRH.

To begin to dissect estrogenic function within the specific regions of the central nervous system, the CRE-lox approach was used and ERα floxed mice were bred to the

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Abbreviations: ADIOL, 5-androsten-3β,17β-diol; DHEA, dehydroepiandrosterone; $ER\alpha^{-/-}$, estrogen receptor α-null mouse; KO, knockout; LPS, lipopolysaccharide; MHT, menopausal hormone therapy; POMC, proopiomelanocortin.

SF1-CRE mice, which allowed us to delete ERα from the ventral lateral portion of the ventral medial nucleus. There was a significant increase in overall body weight in the females but not in the males. The females had a significant deposition of fat in the visceral depot, which was perigonadal adipose tissue. This was the first demonstration deposition of visceral fat due to a brain-specific knockout. Analysis of the adipose tissue demonstrated a significant increase in triglyceride content and an increase in overall lipogenic gene expression, specifically in the visceral/gonadal depot. There was no change in food intake, but a significant reduction in overall energy expenditure that facilitated the increase in body weight. Further analysis of brown adipose tissue in these knockout mice revealed a significant infiltration of white adipocytes into the brown depot, which was associated with a reduction in overall mitochondrial gene expression, in essence a "whitening" of brown adipose tissue. These animals thus had an impairment in overall thermogenesis, probably mediated partially through reduction in sympathetic activity at the level of brown adipose tissue. These findings demonstrated that activity of ERα, specifically in the ventral medial nucleus, is a critical regulator of energy expenditure for females.

To begin to address where ERα might regulate food intake, estrogenic regulation of proopiomelanocortin (POMC) neurons, which are critical for overall food intake and body weight regulation was investigated. We, and others, demonstrated that ERα is colocalized with the POMC neurons. Therefore using the CRE-lox approach, ERα was deleted from POMC neurons. Metabolically characterization of the mice found a significant increase in overall body weight in the females but not in the males. More specifically, in the females, there was a significant increase in food intake and increase in energy expenditure. Importantly, the increase in food intake was greater than the increase in energy expenditure. The female mice also had an increase in overall lean mass. A seminal observation was that these animals had an increase in overall plasma estradiol levels, suggesting that the POMC neuronal population might be important with respect to the negative feedback mechanism consistent with observations of dysregulation of FSH/LH.

Lastly, ERα was knocked out of both POMC and the ventral lateral nucleus neurons. This resulted in an exacerbated phenotype, with an enhanced increase in overall body weight, specifically in the females relative to the wild-type mice or single deletions. There was an accelerated increase in visceral adiposity in the perigonadal depot. Hart-Unger and Korach (3) provided an excellent summary of these findings. Estrogens, specifically acting in the brain, act on the POMC neurons to regulate food intake and the negative feedback loop. When estrogens act on the SF1 neurons, specifically in the ventral medial nucleus, they regulate energy expenditure as well as the deposition of fat within the visceral depot. These are important findings because they suggest estrogens have an important role in the central nervous system to regulate food intake, energy expenditure, body fat distribution, and the reproductive axis.

Gosselin and Rivest discussed (4) sexual dimorphisms with respect to disease processes, specifically, focusing on the heightened sensitivity to autoimmune as well as inflammatory diseases in females. For example, there is a 3- to 5-fold higher incidence of multiple sclerosis in females. In a landmark paper, Glass and colleagues (5) identified a role for estrogens in modulating inflammation. Estrogens and their ligands have long been documented to exert antiinflammatory effects. However, Glass and colleagues (5) focused on the role of microglia and astrocytes, where both ERβ and ERα are highly expressed. Their first observation was that ERβ-specific ligands, based on a halogen-substituted phenyl-2H-indazole-Cl (indazole-Br and indazole-Cl), potently inhibited the transcriptional activation of inflammatory response genes in those cells. They measured this by looking at IL-6, IL-23, and IL-1β. They further demonstrated the ERβ-specific transrepression pathway is controlled endogenously by 5-androsten-3β,17β-diol (ADIOL). Glass and colleagues (5) looked at other markers of inflammation, specifically iNOS, and found a potent effect of the indazoles in suppressing lipopolysaccharide (LPS)-induced iNOS but no effect of the ER modulators on modulating inflammation. They demonstrated these indazoles specifically act through ERβ, both *in vitro* and *in vivo*. Because the indazoles are pharmacological products, they investigated whether endogenous hormones could act in a similar way. They focused their attention on a host of steroid derivatives and found that ADIOL has a similar effects. Specifically, ADIOL has a potent antiinflammatory effect, and they demonstrated that after inflammation there is a significant suppression of ADIOL *in vivo*. Glass and colleagues (5) identified that HSD17B14, which is located in microglia and astrocytes, converts dehydroepiandrosterone (DHEA) to ADIOL, and its expression is controlled by inducers and suppressors of inflammation. So within the microglia, which are critical modulators of inflammation, lies the enzymatic machinery that facilitates the generation of ADIOL. These researchers were also able to demonstrate that the ADIOL component itself is critical for the antiinflammatory effects. Next they demonstrated that the steroid-metabolizing enzyme also is regulated by inflammation. Therefore, in a proinflammatory environment, a reduction in HSD17B14 and in the ADIOL can block an ERβ-dependent antiinflammatory pathway driven by the local

production of endogenous ligands. One of the many interesting findings in this work was that 17β-estradiol itself did not provide a potent antiinflammatory effect. Glass and colleagues (5) examined whether 17β-estradiol combats the beneficial effect of ADIOL. In every case, increasing 17β-estradiol levels decreased the ability of ADIOL to provide an antiinflammatory effect. They posited the following mechanism: An inflammatory stimulus, perhaps LPS, activates the TLR4 cascade, inducing c-Fos as well as c-Jun. Induction of protein kinase A (PKA) induces a phosphorylation of ERβ. ERβ tethers to c-Jun/c-Fos, and with conversion of DHEA to ADIOL, ERβ is activated causing a transrepression effect that inhibits the inflammatory cascade. Importantly, in males, who have relatively low levels of 17β-estradiol, this would enable DHEA to be converted to ADIOL and provide a potent antiinflammatory effect. These findings may explain why men have much lower incidences of inflammatory diseases than women. Furthermore, the authors posited that 17β-estradiol may inhibit the beneficial effects of ERβ to cause transrepression of the inflammatory cascade.

Other important studies have assessed the impact of estrogens in the periphery on inflammation. Hevener and colleagues (6) knocked out ERα from myeloid-specific cells and found that hematopoietic or myeloid-specific deletion of ERα caused the metabolic syndrome. Specific alterations resulted in increased adipokine and cytokine levels, glucose intolerance with increased insulin resistance, and increased adiposity. Transplanting $ER\alpha^{-/-}$ bone marrow into low-density lipoprotein receptor-KO (LDLR-KO) mice resulted in an obese phenotype with an increased level of atherosclerotic lesions. These researchers also demonstrated that in isolated macrophages, ERα was critical for the antiinflammatory response mediated through repression of inflammation, maintenance of oxidative metabolism, and IL-4-mediated induction of activation and phagocytic response to LPS. Finally, they demonstrated that ERα is a direct regulator of transglutaminase 2 expression in macrophages. These important findings demonstrate that knocking out ERα from macrophages produces a metabolic phenotype characterized by unhealthy adipose tissue rimmed with crown-like structures and increased markers of inflammation. This is associated with insulin resistance in the skeletal muscle and pancreas and hepatic insulin resistance with a significant increase in atherosclerotic plaque in the arteries.

In a further attempt at understanding the sites and mechanisms of estrogenic action, Park *et al.* (7) set out to examine whether estrogenic activity to regulate body weight homeostasis is through the classical or the nonclassical ER signaling pathways. ERs signal through either nuclear ERs (classical or genomic signaling) or through rapid induction of intracellular signaling cascades such as phosphorylated phosphatidylinositol-3 kinase (PP13K) or Akt as well as MAPK (nonclassical or nongenomic signaling). Park and colleagues (7) therefore determined the impact of blocking estrogen's ability to bind to the estrogen response element with respect to body weight homeostasis. To do this, they used a mouse model that is a knock-in with a blocker of the DNA-binding domain of ERα, which inhibits ERα's ability to bind to the estrogen response element. These mice, termed NERKI (nuclear ERα knock-in) were subsequently metabolically characterized. The NERKI mice were leaner and had normal glucose homeostasis, insulin sensitivity, energy homeostasis, and physical activity when compared with $ER\alpha^{-/-}$ or wild-type mice. The NERKI mice had lower leptin levels than $ER\alpha^{-/-}$ and enhanced brain-specific leptin sensitivity as measured by phospho-STAT3 ($pSTAT_3$). The authors found an increase in phosphorylated Akt after 17β-estradiol injections in the ventral medial nucleus, which, as previously discussed, is a critical site of estrogenic action for energy homeostasis. Finally, the authors demonstrated, despite normalization of the body weight, the mice had a reproductive phenotype. So although nonclassical signaling appears to be very important with respect to metabolism, it appears that genomic/classical estrogenic action is required for fertility.

Tiano and colleagues (8) examined the role of estrogen in pancreatic β-cells based on their observations that in many rodent models of diabetes and dysregulated glucose homeostasis, the females remained relatively protected with respect to the pancreatic β-cell function. Tiano *et al.* (8) posited that ovarian hormones may provide protection against pancreatic β-cell abnormalities. These authors focused on what happens in males when supplemented with estrogens. In the first series of experiments, the authors found that treating male Zucker diabetic fatty rats with 17β-estradiol suppressed the synthesis and accumulation of fatty acids and protected against pancreatic β-cell failure. Furthermore, they demonstrated that the antilipogenic actions of estrogens were replicated with pharmacological activation of ERα. The authors looked at a host of glucose parameters and in every case found that estrogenic supplementation reversed the effects in the dysregulated glucose homeostasis in diabetic fatty rats. In addition, deletion of ERα from the pancreas prevented the reduction of lipid synthesis by estrogens and increased islet lipid accumulation and β-cell dysfunction in response to a high-fat diet. The authors demonstrated that estrogenic activation inhibited β-cell lipid synthesis by suppressing the expression of fatty acid synthase through a nonclassical (NERKI) pathway that was dependent on activated ($pSTAT_3$) STAT3. Finally, they demonstrated that deletion of STAT3 from the pancreas curtailed ERα-mediated suppression of lipid synthesis.

In a series of experiments looking at circulating free fatty acids, they found when male animals received 17β-estradiol, there was a significant increase in overall circulating free fatty acid levels, with a significant suppression of fatty acids in the pancreatic islet cells. So there appears to be a dichotomy between the circulating fatty acid levels and estrogenic action at the level of the islet cells. To begin to address this, Tiano and colleagues (8) then examined whether suppression of fatty acids in the pancreatic β-cell occurs with all estrogenic compounds. Previously, the authors had demonstrated that ERα, ERβ, and GPR30 are located in the pancreatic islet cells. They therefore used specific agonists to those ERs/GPR30 and examined whether triglyceride content was suppressed. In another experiment with mice in which these ERs/GPR30 had been deleted from pancreatic β-cells, they showed that pulses with estrogen produced no beneficial effect. In addition, they showed that exposing these animals to a high-fat diet exacerbated the increase in triglyceride content. They then looked at the pathway through which the beneficial effect on pancreatic islet cells occurs and, using the NERKI mouse, showed that the nonclassical estrogenic pathway is critical for the suppression of fatty acids.

Many of the new findings relating metabolic effects of ERs in pancreatic β-cells, adipose tissue, and skeletal muscle were nicely summarized in a review from Barros and Gustafsson published in 2011 (9).

In conclusion, it is worth noting that studying sex-based differences is an expensive, complex undertaking. It requires determining which phase of the estrogenic cycle females are in to enhance the understanding of the complex role of female gonadal steroids. Many publications lump women of all ages into one classification, yet women transition from cycling to perimenopausal to menopausal with differing levels of hormones and hormonal action at each of these phases of life. Currently, there is a state of uncertainty as to how to approach hormonal treatment and supplementation in women who are in the menopausal transition. In recommendations for the diagnosis and treatment of menopause from the American Association of Clinical Endocrinologists (AACE) (10), they specifically note that menopausal hormone therapy (MHT) may be appropriate for women at different life stages and by different routes of administration. Here we highlight important new findings that estrogen's beneficial metabolic effects occur at critical sites of action in a variety of tissues. It will therefore be challenging to devise specific targeted therapies for addressing metabolic issues for women as they transition through menopause and to specifically deliver estrogens where they need it to be targeted. The goal of MHT, according to the AACE, is to alleviate the quality-of-life symptoms in menopausal and perimenopausal women. The AACE recognizes that chronic disorders associated with aging and the menopausal state affect the brain, skeleton, and urogenital and cardiovascular systems. That said, it is important to remember that the role of MHT in the prevention of such disorders remains controversial. With all of the new literature focusing on where and how estrogens modulate homeostasis, we will need to continue to challenge ourselves to develop novel therapies for women that alleviate the deleterious estrogenic effects and promote the beneficial effects of estrogens.

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