Altered estrogen receptor expression in skeletal muscle and adipose tissue of female rats fed a high-fat diet

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Gorres BK, Bomhoff GL, Gupte AA, Geiger PC. Altered estrogen receptor expression in skeletal muscle and adipose tissue of female rats fed a high-fat diet. J Appl Physiol 110: 1046–1053, 2011. First published January 13, 2011; doi:10.1152/japplphysiol.00541.2010.—Estrogen receptors (ERs) are expressed in adipose tissue and skeletal muscle, with potential implications for glucose metabolism and insulin signaling. Previous studies examining the role of ERs in glucose metabolism have primarily used knockout mouse models of ERα and ERβ, and it is unknown whether ER expression is altered in response to an obesity-inducing high-fat diet (HFD). The purpose of the present study was to determine whether modulation of glucose metabolism in response to a HFD in intact and ovariectomized (OVX) female rats is associated with alterations in ER expression. Our results demonstrate that a 6-wk HFD (60% calories from fat) in female rats induces whole body glucose intolerance with tissue-specific effects isolated to the adipose tissue, and no observed differences in insulin-stimulated glucose uptake, GLUT4, or ERα protein expression levels in skeletal muscle. In chow-fed rats, OVX resulted in decreased ERα with a trend toward decreased GLUT4 expression in adipose tissue. Sham-treated and OVX rats fed a HFD demonstrated a decrease in ERα and GLUT4 in adipose tissue. The HFD also increased activation of stress kinases (c-Jun NH2-terminal kinase and inhibitor of κB kinase β) in the sham-treated rats and decreased expression of the protective heat shock protein 72 (HSP72) in both sham-treated and OVX rats. Our findings suggest that decreased glucose metabolism and increased inflammation in adipose tissue with a HFD in female rats could stem from a significant decrease in ERα expression.

A recent study by Ribas et al. (37) further showed that ERα expression is critical for the maintenance of whole body insulin action and protection against tissue inflammation in response to high-fat feeding. These investigators suggest that ERα could play an important role in modulating inflammatory stress kinase proteins such as c-Jun NH2-terminal kinase (JNK) (37), known to interfere with insulin signaling (9, 16, 17, 37). Despite this important new information, the role of ERs in the pathogenesis of insulin resistance and glucose intolerance is not clear. Previous studies examining the role of ERs in glucose metabolism have primarily used knockout mouse models of ERα and ERβ and it is unknown whether ER expression is altered in response to an obesity-inducing high-fat diet (HFD). As a result, the impact of a HFD on ER expression in adipose tissue and skeletal muscle, and thus the role of ERs in mediating the metabolic actions of estrogen, remains a fundamental question. Therefore, the purpose of the present study was to determine whether modulation of glucose metabolism in response to a HFD in intact and ovariectomized (OVX) female rats is associated with alterations in ER expression.

MATERIALS AND METHODS

Materials. GLUT4 antibody (ab654) was purchased from Abcam (Cambridge, MA), ERα (MC-20) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), ERβ (PA1-310B) was purchased from Affinity BioReagents (Rockford, IL), and HSP72 was purchased from Stressgen (Victoria, BC, Canada). Phospho-SAPK/JNK (T183/Y185), total SAPK/JNK, and IkBα were purchased from Cell Signaling (Beverly, MA). Goat anti-mouse HRP-conjugated secondary antibody was obtained from Bio-Rad (Hercules, CA) and donkey anti-rabbit HRP-conjugated secondary antibody was purchased from Jackson (West Grove, PA). Enhanced chemiluminescence reagents were purchased from Fisher Scientific (Pittsburgh, PA). [14C]mannitol and 2-deoxy-[1,2-3H]glucose were purchased from American Radiolabeled Chemicals (St. Louis, MO). All other reagents were obtained from Sigma.

Experimental animals and treatment. Female Sprague Dawley rats (5 mo old) were purchased from Charles River Laboratories (Wilmington, MA) and singly housed in a temperature-controlled (22 ± 2°C) room with 12:12-h light-dark cycles. Chow rats were fed ad libitum on a soy protein-free diet (Harlan Teklad 2020X, Madison, WI, 10% calories from fat), whereas HF rats received a modified Kreagen diet (43) of 60% calories from fat for 6 wk as previously used (16), which contains the following: 254 g/kg casein, 85 g/kg sucrose, 169 g/kg cornstarch, 11.7 g/kg vitamin mix, 1.3 g/kg choline chloride, 67 g/kg mineral mix, 51 g/kg bran, 3 g/kg methionine, 19 g/kg gelatin, 121 g/kg corn oil, 218 g/kg lard. A preset amount of food (in excess of what was needed) was administered to each animal. The remaining food was weighed 2–3 days later, before giving a new batch of food. At the start of the diet, animals underwent ovariectomy (OVX) or sham surgery under ketamine-atropine-xylazine anesthesia (60 mg/kg body wt ketamine, 0.4 mg/kg body wt atropine, 8 mg/kg body wt xylazine). Bilateral flank incisions were made under aseptic conditions. The ovaries were identified and either bilaterally removed via

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Type 2 diabetes, one of the main causes of morbidity and mortality worldwide (40), is characterized by insulin resistance, glucose intolerance, and inflammation and is closely associated with obesity. Clinical evidence suggests postmenopausal women have an increased risk of glucose intolerance and weight gain and this is accompanied by increased inflammation and decreased insulin sensitivity (7, 33, 42). Estrogen replacement therapy in postmenopausal women ameliorates the increased risk of type 2 diabetes (2, 25, 28), even in the presence of increased abdominal fat (14). While this beneficial effect of estrogen is evident, the molecular mechanisms of estrogen and its active metabolite, 17β-estradiol (E2), in metabolic tissue remain unknown.

Estrogen exerts its effects through two nuclear receptors, estrogen receptor (ER)α and ERβ (11). ERα and ERβ are expressed in adipose tissue and skeletal muscle, with potential implications for glucose metabolism and insulin signaling. Previous studies demonstrate that ERα knockout mice are obese, insulin resistant, and exhibit glucose intolerance (5, 20).
cauterization (OVX) or left intact (sham). Wounds were closed using sutures and wound clips. The following four groups were assessed (n = 5–6 rats/group): 1) chow sham; 2) chow OVX; 3) HF sham; and 4) HF OVX. All protocols were approved by the Animal Care and Use Committee of the University of Kansas Medical Center.

**Intraperitoneal glucose tolerance test.** An intraperitoneal glucose tolerance test (IPGTT) was performed during week 6 of the diet regimen. Overnight-fasted rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (2.5 mg/100 g body wt) and given a glucose load of 2 g/kg body wt in 0.9% saline. Tail blood samples were measured with a glucometer (Accu-Check) at time points 0, 15, 30, 60, 90, and 120 min after glucose injection. Serum insulin was measured via an ELISA according to the manufacturer’s instructions (Alpco Diagnostics, 80-INSRT-E01; Salem, NH).

**Tissue dissection.** During week 7, overnight-fasted animals were anesthetized under ketamine-atropine-xylazine anesthesia (60 mg/kg body wt ketamine, 0.4 mg/kg body wt atropine, 8 mg/kg body wt xylazine). One soleus and one extensor digitorum longus (EDL) muscle was dissected from each animal, each split longitudinally into strips, and assessed for glucose transport. The remaining soleus and EDL muscle from each animal was frozen in liquid nitrogen for Western blot analysis. Gonadal fat was removed from the ovaries and uterine horns, weighed, and then frozen in liquid nitrogen. The uterus was also removed and weighed.

**Measurement of glucose transport activity.** Glucose transport was measured in soleus and EDL muscle strips as previously described (17). Muscle strips recovered for 60 min in flasks containing 2 ml of Krebs-Henseleit bicarbonate buffer (KHB) with 8 mM glucose, 32 mM KCl, and 0.4 mM phenylmethylsulfonyl fluoride; 2 mM Na3VO4; 20 mM β-glycerophosphate; 5–25 μM brefeldin A; and 100 μg/ml calpain inhibitor II (Calbiochem, Darmstadt, Germany). The muscle strips were then blotted dry, clamp frozen in liquid nitrogen, and weighed. The remaining soleus and EDL muscle from each animal was frozen in liquid nitrogen for Western blot analysis. Gonadal fat was removed from the ovaries and uterine horns, weighed, and then frozen in liquid nitrogen. The uterus was also removed and weighed.

**Results**

**Effects of diet and OVX on food intake and body composition.** Uterine weight is a commonly used bioassay to assess in vivo estrogen status. In the current study, OVX rats had significantly lower uterine weight compared with sham-treated rats (0.415 ± 0.016 vs. 1.618 ± 0.108 mg/g body wt; P < 0.001), confirming negligible estrogen influence in OVX rats as a result of surgically removing the ovaries. To confirm the in vivo estrogen status, serum E2 levels were also measured. OVX significantly decreased serum E2 levels compared with sham-treated animals (7.6 ± 0.9 vs. 11.9 ± 1.8 pg/ml; P < 0.05), which is consistent with previous reports in the literature (18, 22). The 6-wk HFD did not significantly alter uterine weight or serum E2 levels in either OVX or sham-treated animals, consistent with previously reported data (1, 6). Over the course of the 6-wk diet regimen, female rats that underwent OVX demonstrated greater average daily food intake and increased body weight compared with sham-treated rats, with no significant difference in food intake or weight gain as a result of the HFD in sham-treated rats (Fig. 1, A and B). OVX animals fed a HFD demonstrated greater food intake and weight gain compared with all other groups. Both groups of HFD animals gained most of their weight in first 2–3 wk of high-fat feeding. However, the body weight of the animals that underwent OVX and were fed a HFD increased at a greater rate during this period. After week 3, the OVX animals fed the HFD continued to gradually increase their body weight, and the sham-treated animals fed the HFD remained fairly constant. Assessment of fat mass, as measured by gonadal fat pad weight, revealed a different pattern from that observed for food intake and body weight. Despite increased food intake and body weight as a result of OVX, no increase in gonadal fat was observed in this group (Fig. 1C). The HFD resulted in a significant increase in gonadal fat pad weight in both sham- and OVX-treated rats.

**Effects of diet and OVX on glucose tolerance.** Fasting glucose and insulin levels did not differ across experimental groups at the end of the 6-wk diet (Fig. 2, A and B, respectively). An intraperitoneal glucose tolerance test (IPGTT) was performed to assess whole body glucose clearance in response to a glucose challenge. The HFD resulted in a decrease in whole body glucose tolerance, as demonstrated by the inability of HFD rats to effectively clear glucose from their blood by the end of the 2-h test, compared with rats fed a Chow diet (Fig. 2C). While OVX rats fed a HFD had slightly lower glucose values throughout the test, these values were not significantly different from sham-treated animals fed a HFD. Similarly, OVX did not significantly alter glucose clearance in Chow-fed rats compared with sham controls. Serum insulin levels during the IPGTT did not differ among the groups (data not shown).
Effects of diet and OVX on insulin-stimulated skeletal muscle glucose uptake. To investigate the effects of diet and OVX on skeletal muscle glucose uptake, we performed 2-DG uptake assays on the predominately slow-twitch soleus or the predominately fast-twitch extensor digitorum longus (EDL) muscles. Insulin-stimulated glucose uptake increased above basal in all groups examined (Fig. 3, A and B, respectively). However, no differences in basal- or insulin-stimulated skeletal muscle glu-

Fig. 2. A 6-wk HFD decreases glucose tolerance in female rats. At the end of the 6-wk study, body weight (B) and gonadal fat weight (C) were measured. Values are means ± SE for 5–6 rats per group. *P < 0.05 vs. chow sham; +P < 0.05 vs. HF sham; #P < 0.05 vs. chow OVX.

Fig. 3. A 6-wk HFD increases food intake, body weight, and gonadal fat weight. Average daily food intake was measured over the course of a 6-wk chow or HFD (A). At the end of the 6-wk study, body weight (B) and gonadal fat weight (C) were measured. Values are means ± SE for 5–6 rats per group. *P < 0.05 vs. chow sham; +P < 0.05 vs. HF sham; #P < 0.05 vs. chow OVX.
Cose uptake were observed across treatment groups in either the soleus or EDL muscles.

**Effects of diet and OVX on ERα, ERβ, and GLUT4 protein levels.** The effects of high-fat feeding on ER protein expression in metabolic tissue have not been previously examined in nontransgenic animal models. ERα and ERβ are both prevalent in skeletal muscle and adipose tissue, with ERα expression shown to be more highly expressed than ERβ in insulin-sensitive tissue (37). Neither OVX nor the HFD had an effect on ERα expression in the soleus or EDL muscles (Fig. 4, A and B). However, ERα expression was significantly decreased in adipose tissue in response to OVX and the HFD (Fig. 4C). In OVX rats fed a HFD, the decrease in ERα was not greater than with either intervention alone. In contrast with ERα expression, there was an effect of OVX and diet on ERβ expression in skeletal muscle, but these effects were isolated to the soleus muscle. In this muscle, OVX and a HFD resulted in significant decreases in ERβ expression compared with sham controls (Fig. 4D). The combination of a HFD with OVX did not result in a greater decrease in ERβ expression in soleus muscle, and no changes with OVX or diet were observed in the EDL muscle (Fig. 4E). In the adipose tissue, ERβ expression was unchanged by OVX or a HFD (Fig. 4F).

The effect of a HFD on GLUT4 protein expression is equivocal with some studies demonstrating a decrease or no change in GLUT4 protein expression as a result of high-fat feeding (19, 24, 26, 39, 41, 44, 47). In female rats subject to OVX or sham surgery, a 6-wk HFD had no effect on GLUT4 protein expression in either the soleus or EDL muscle (Fig. 5, A and B). However, the HFD dramatically reduced GLUT4 protein expression in adipose tissue in both sham-and OVX-treated rats (65% and 52%, respectively; Fig. 5C). OVX in chow-fed rats resulted in lower GLUT4 levels in adipose tissue...
compared with sham-treated chow rats, although these differences were not statistically significant ($P = 0.07$).

Effects of diet and OVX on stress kinases and HSP72 protein levels. Activation of the stress kinases c-Jun NH$_2$-terminal kinase (JNK) and inhibitor of κB kinase β (IKKβ) was assessed via Western blot analysis. JNK activation was assessed by measuring changes in JNK protein phosphorylation and IKKβ by protein levels of IκBα, the downstream protein targeted for degradation by IKKβ. JNK phosphorylation was increased as a result of the HFD in adipose tissue (Fig. 6A), but no change in JNK phosphorylation occurred in either soleus or EDL muscle in response to diet (data not shown). JNK phosphorylation with OVX treatment alone or in combination with a HFD was not different from that in chow-fed sham animals in adipose tissue or skeletal muscle. Activation of IKKβ was also increased with the HFD in adipose tissue, as indicated by decreased expression of IκBα (Fig. 6B). No changes in adipose tissue IκBα protein expression occurred with OVX in either chow or high fat-fed rats. In addition, no changes were observed in IκBα expression in either the soleus or EDL muscle as a result of diet or OVX (data not shown). Six weeks of a HFD dramatically decreased protein levels of heat shock protein 72 (HSP72) in the adipose tissue of both sham-treated and OVX rats (Fig. 6C). OVX alone had no effect on protein levels of HSP72 in adipose tissue. Neither the HFD nor OVX resulted in alterations in HSP72 protein expression in the soleus or EDL muscles (data not shown).

DISCUSSION

The purpose of the current study was to examine the effects of a HFD on adipose tissue and skeletal muscle glucose metabolism in female rats with and without OVX and to determine whether modulation of glucose metabolism in response to a HFD could be attributed to alterations in ER expression. While a short-term HFD in female rats induced whole body glucose intolerance, tissue-specific effects were isolated to the adipose tissue with no observed differences in insulin-stimulated glucose uptake, GLUT4, or ERα protein expression levels in skeletal muscle. GLUT4 protein decreased dramatically in adipose tissue of OVX and sham-treated rats as a result of a HFD, as did expression of ERα, the ER isoform previously shown to positively mediate glucose metabolism (3, 4, 29). Increased stress kinase activation and decreased HSP72 expression in adipose tissue in response to a HFD further demonstrates the impact of high-fat feeding on this tissue. These new findings highlight the differential effects of high-fat feeding in female compared with male rats, with previous studies demonstrating a significant decrease in skeletal muscle glucose metabolism in response to a HFD in male rats (16, 19, 32, 44, 47). In addition, our findings suggest a high-fat diet-induced loss of ERα in adipose tissue may be a contributing factor in the pathogenesis of glucose intolerance in female rats.

Ribas et al. (37) recently showed that female ERα knockout mice have decreased whole body glucose tolerance compared with wild-type mice, suggesting that the absence of ERα results in decreased glucose metabolism. While these data indicate that ERα is critical for the maintenance of whole body insulin action, the effect of a HFD on ER expression in insulin responsive tissue was unknown. Our findings reveal that ERα expression was decreased with a HFD only in the adipose
tissue, which also displayed decreased GLUT4 protein and likely reflects lower glucose utilization in this tissue. OVX animals fed a chow diet demonstrated decreased ERα without corresponding changes in GLUT4, whole body glucose tolerance, or markers of inflammation. While the role of ERα in mediating glucose metabolism cannot be firmly established from these data, these findings suggest ERα-mediated effects may be dependent on additional changes induced by the HFD (stress kinase activation and HSP expression changes). In contrast, in the insulin-responsive skeletal muscle tissue, ERα expression was unchanged as was glucose uptake and GLUT4 protein expression levels. It is still possible that in insulin-resistant skeletal muscle (such as that from male rats fed a HFD), alterations in ERα expression could occur and contribute to changes in glucose metabolism. Other data support our findings of an adipose tissue-specific effect of the HFD in female rats. For example, Riant et al. (36) demonstrated that the combination of a HFD and OVX resulted in decreased glucose utilization in adipose tissue with no changes in soleus or EDL muscles in female mice (these investigators did not assess ER expression changes). Our findings of decreased ERα in adipose tissue in the current study support the idea that ERα is the primary functioning ER in adipose tissue (3). In turn, ERβ has been suggested as the primary functioning ER in skeletal muscle (3), which is coincident with our findings of decreased ERβ in the soleus muscle in response to a HFD. Decreased ERβ, the ER isoform suggested to have a suppressive role on GLUT4 expression (4), could result in protection from HFD-induced insulin resistance in skeletal muscle. The effects of estrogen on skeletal muscle likely depend on the balance between the two receptors, and future studies are needed to determine the regulatory roles of ERs in skeletal muscle.

Barros et al. (3, 4) have previously shown that ERs modulate GLUT4 expression in adipose tissue and skeletal muscle. Although the potential mechanism has yet to be demonstrated in skeletal muscle, ERα could modulate GLUT4 expression through specificity protein 1 and nuclear factor-κB. Ribas et al. did not find a decrease in skeletal muscle GLUT4 expression in ERα knockout mice despite insulin resistance and decreased glucose uptake in these mice (37). As these investigators point out, GLUT4 expression is regulated by redundant transcriptional pathways and ERα is likely only one of these pathways. However, our findings, and others (3) seem to suggest that ERα modulation of GLUT4 occurs primarily in the adipose tissue, and future studies will be needed to assess transcriptional control of GLUT4 by ERα in adipose tissue.

Estrogen has the potential to regulate fat storage and triacylglyceride accumulation by altering transcription of lipogenic proteins such as SREBP-1 and its downstream targets, ACC, and FAS (5, 8, 10, 23, 31). The effects of estrogen on lipogenic pathways have primarily been assessed in response to...
estrogen treatment or replacement. For example, Phraonkham et al. (34) demonstrated that estrogen treatment increased FAS expression in cultured adipocytes. However, other studies have demonstrated opposite effects, with estrogen treatment in mice shown to decrease ACC and FAS mRNA in adipose tissue (6, 10). As has been previously shown, physiological estrogen levels may positively modulate glucose metabolism while high or low estrogen levels have a different effect (29, 30). More studies are needed to assess the role of estrogen and ER expression in modulating lipogenic pathways in cycling, OVX, and estrogen-treated animals.

Increased lipid intermediates and oxidative stress in insulin-responsive tissues can result in activation of stress kinases (16, 17, 21, 38, 46). We (16, 17) and others (9, 37) have previously shown that increased stress kinase activation and decreased HSP expression contribute to decreased insulin signaling and glucose uptake in skeletal muscle. Further evidence suggests that ERα may be involved in stress kinase activation and HSP expression. Ribas et al. demonstrate increased activation of JNK in skeletal muscle and adipose tissue of ERα knockout mice (37). When challenged with a HFD, ERα knockout mice display greater JNK activation and decreased HSP72 expression in adipose tissue compared with high-fat-fed wild-type mice (37). These data suggest that ERα may contribute to glucose regulation by positively modulating stress kinase activation and HSP expression. Evidence of inflammation, increased stress kinase activation (increased pJNK and decreased IκBα), and decreased HSP72 expression in adipose tissue were observed in the present study, although these changes did not always correlate with changes in ERα levels. With OVX alone, ERα protein was decreased in adipose tissue without changes in inflammation observed with a HFD (increased stress kinases and decreased HSP72). As OVX alone did not result in increased adiposity or glucose intolerance, it is possible the combination of decreased ERα and increased inflammation, as observed with the HFD, is critical for glucose intolerance. These results indicate the complex interplay of diet, hormones, and inflammation in insulin-responsive tissue require further investigation.

While this study focuses on the effect of a HFD and OVX on adipose tissue and skeletal muscle glucose metabolism, the liver is also an important regulator of glucose metabolism. In ERα knockout mice, modest hepatic insulin resistance is present as demonstrated by elevated hepatic glucose production during insulin stimulation and decreased insulin receptor substrate-PI 3-kinase p85 association compared with wild-type mice (37). Plausibly, ERα knockouts could have impaired signal transducer and activator of transcription 3 (STAT3) function. Estrogen treatment upregulates STAT3, which suppresses key enzymes in glucose homeostasis, including the gluconeogenic genes glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) (12, 27, 35). Future studies are needed to assess the effects of OVX and a HFD on ER protein expression in the liver and their role in regulating hepatocyte substrate metabolism.

While previous studies demonstrate the importance of the ERs in regulating glucose metabolism, the impact of a HFD on ER expression in skeletal muscle and adipose tissue was unknown. Findings from the present study indicate a short-term HFD in female rats induced whole body glucose intolerance, along with decreased ERα and GLUT4 in adipose tissue. In contrast with previous findings using male rodents, a short-term HFD did not decrease skeletal muscle glucose uptake in female rats. In addition, decreased ERβ expression was observed in the soleus muscle with no changes in skeletal muscle ERα expression. Future studies are needed to determine the tissue-specific regulation of ERs and how altered ER expression and/or function may contribute to increased susceptibility to type 2 diabetes.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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