Estrogens Protect against High-Fat Diet-Induced Insulin Resistance and Glucose Intolerance in Mice

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Although corroborating data indicate that estrogens influence glucose metabolism through the activation of the estrogen receptor (ER), it has not been established whether this pathway could represent an effective therapeutic target to fight against metabolic disturbances induced by a high-fat diet (HFD). To this end, we first evaluated the influence of chronic 17β-estradiol (E2) administration in wild-type ovariectomized mice submitted to either a normal chow diet or a HFD. Whereas only a modest effect was observed in normal chow diet-fed mice, E2 administration exerted a protective effect against HFD-induced glucose intolerance, and this beneficial action was abolished in ERα-deficient mice. Furthermore, E2 treatment reduced HFD-induced insulin resistance by 50% during hyperinsulinemic euglycemic clamp studies and improved insulin signaling (Akt phosphorylation) in insulin-stimulated skeletal muscles. Unexpectedly, we found that E2 treatment enhanced cytokine (IL-6, TNF-α) and plasminogen activator inhibitor-1 mRNA expression induced by HFD in the liver and visceral adipose tissue. Interestingly, although the proinflammatory effect of E2 was abolished in visceral adipose tissue from chimeric mice grafted with bone marrow cells from ERα-deficient mice, the beneficial effect of the hormone on glucose tolerance was not altered, suggesting that the metabolic and inflammatory effects of estrogens can be dissociated. Eventually comparison of sham-operated with ovariectomized HFD-fed mice demonstrated that endogenous estrogens levels are sufficient to exert a full protective effect against insulin resistance and glucose intolerance. In conclusion, the regulation of the ERα pathway could represent an effective strategy to reduce the impact of high-fat diet-induced type 2 diabetes. (Endocrinology 150: 2109–2117, 2009)

Lifestyle evolution, and precisely the spreading of fat-enriched diet, largely contributes to the worldwide growing incidence of type 2 diabetes and cardiovascular diseases (1). Supporting its causal role in these pathophysiological processes, high-fat feeding was demonstrated in animal models to trigger insulin resistance, one of the major features of the metabolic syndrome (2, 3). Thus, in addition to the classical preventive strategy based on physical activity and reduced calorie intake, the identification of new molecular targets able to avoid, or at least to limit, insulin resistance and the metabolic disturbances induced by such a hyperlipidic nutritional stress actually represents one of the most important public health challenge.

During the last decades, corroborating data from clinical and experimental studies revealed that, besides their pivotal role in sexual development and reproduction, estrogens contribute to glucose homeostasis (4). Supporting the beneficial effect of estrogens on insulin action and glucose homeostasis, insulin sensitivity was demonstrated to be higher in women before the menopause than in age-matched men (5, 6). In addition, it is well recognized that the menopause favors visceral fat deposition and insulin resistance, leading to a significant increase in type 2 diabetes risk (7). In addition, hormonal replacement therapy was recently reported to reduce the incidence of type 2 diabetes in postmenopausal women. In randomized placebo-controlled...
studies, a significant 21–35% decrease in diabetes occurrence was observed in menopausal women receiving the association of conjugate equine estrogens and medroxyprogesterone acetate (8, 9). Furthermore, in animal models, namely monkeys and rodents, the bilateral ovariectomy of females was shown to impair insulin sensitivity and glucose metabolism, a deleterious metabolic effect reversed by the chronic administration of estrogens (10, 11). However, to the best of our knowledge, except one report in the specific model of partially pancreatectomized rats (12), all of these in vivo studies were conducted with animals fed a normal chow diet.

The specific effect of estrogens and their signaling pathways in the regulation of insulin action and glucose metabolism has been further illustrated by the demonstration that subjects bearing genetic mutations, which lead to the lack of either estrogen receptor (ER)-α or aromatase expression, develop visceral adiposity, insulin resistance, and impaired glucose tolerance (13, 14). Genetically engineered mice models have confirmed these clinical observations because either ERα or aromatase gene invalidation similarly favors several features of the metabolic syndrome (15–17). Altogether, these data indicate that endogenous estrogens, through the activation of ERα, exert a beneficial effect on insulin sensitivity and glucose tolerance. However, whether the administration of exogenous estrogens would represent an effective strategy to fight against high-fat diet-induced insulin resistance and glucose intolerance in wild-type mice remains to be determined.

The mechanisms by which estrogens influence insulin sensitivity and glucose metabolism remain poorly understood but could be related to their role on the control of the innate immune system (18). This hypothesis is supported by the fact that impaired glucose tolerance and type 2 diabetes are characterized by a low-grade inflammatory state (2). Several cytokines, mostly derived from the adipose tissue, have been demonstrated to directly impair the insulin signaling pathway in experimental models and hence play a role in the regulation of insulin resistance (19, 20). Along the same line of investigation, the deleterious metabolic effect of high-fat diet in mice, leading to the initiation of insulin resistance and glucose intolerance, has been recently demonstrated to be mainly mediated through the induction of inflammatory cytokine expression in adipose tissues and the liver (3). However, although estrogens are well recognized to exert regulatory functions on the immune system and the expression of cytokines (18), the hypothesis that these hormones could interfere with the early inflammatory response observed in animal models of insulin resistance and type 2 diabetes has not been addressed to date.

Thus, in the present work, we first evaluated the therapeutic potential of chronic 17β-estradiol (E2) administration to control insulin resistance and glucose intolerance in ovariectomized mice fed a high-fat diet. We then addressed the question whether the metabolic effect of the hormone was mediated through ERα activation and was associated with improved insulin signaling and/or with changes in the early inflammatory immune response induced by the nutritional stress. Finally, we examined the influence of endogenous estrogens levels on insulin sensitivity and glucose tolerance and concluded that the estrogens pathway represents an effective target to protect against high-fat diet-induced metabolic disturbances.

Materials and Methods

Animals and experimental procedures

Four-week-old C57BL/6J female mice were purchased from Charles River (L’Arbresle, France). ERα-deficient (ERα−/−) mice were bred in our animal facilities as previously described (21). Mice were housed in a specific pathogen-free controlled environment. Female mice were either ovariectomized or sham-operated at 4 wk of age (22). To study the effect of chronic E2 administration, ovariectomized mice were then s.c. implanted with pellets releasing either placebo or E2 (0.1 mg for 60 d, i.e., 80 μg/kg·d; Innovative Research of America, Sarasota, FL) at 5 wk of age [except in bone marrow (BM) transplantation experiments, 10 wk of age]. In all experiments, the efficacy of E2 treatment was systematically assessed after the animals were killed by the induction of uterus hypertrophy contrasting with a total atrophy in placebo-treated ovariectomized female mice.

According to the experimental settings, mice were fed with either a normal chow diet (NCD; energy content: 12% fat, 28% protein, and 60% carbohydrate; A04, Villemoisson sur Orge, France) or a high-fat carbohydrate-free diet (HFD) for 4 or 12 wk. The HFD contained 72% fat (corn oil and lard), 28% protein, and less than 1% carbohydrate, as described (3). Animal experimental procedures were all validated by the local animal ethical committee.

Bone marrow transplantation

Six-week-old ovariectomized C57BL/6J female mice were subjected to 9.5-Gy lethal total body γ-irradiation as described (22). Twelve hours later, they were iv reconstituted with bone marrow cells (5 × 106) extracted from the femur and tibia of either ERα+/+ or ERα−/− female mice. Mice were then allowed to recover for 4 wk under the NCD. Then mice were implanted with placebo or E2 pellets and were switched to the HFD for 4 wk.

Intraperitoneal glucose tolerance test (IPGTT)

An ip injection of glucose (IPGTT, 1 g/kg body weight) was performed in 6-h-fasted mice after 4 and 12 wk of diet regimen. Blood glucose levels were monitored with a glucose meter (Roche Diagnostic, Grenoble, France) at 30, 0, 30, 60, and 90 min after glucose injection.

In vivo glucose use rate

To determine the rate of glucose use, an indwelling catheter was placed into the femoral vein of anesthetized mice, sealed under the back skin, and glued onto the top of the skull. The mice were allowed to recover for 4 to 6 d as described by Cani et al. (3). Then mice were fasted for 6 h, and the whole body glucose use rate was determined in hyperinsulinemic euglycemic conditions, as previously described (23).

In different sets of mice, to determine an index for the individual tissue glucose use rate, a flash injection of 30 μCi per mouse of D-2-[3H]deoxyglucose (PerkinElmer, Courtaboeuf, France) through the femoral vein was performed 60 min before the end of the infusions. Plasma D-2-[3H]deoxyglucose disappearance and glucose concentration were determined in 5-μl drops of blood sampled at 0, 5, 10, 15, 20, 25, 30, 45, and 60 min after the injection.

Isotope measurements

For glucose turnover measurements, D-[3-3H]glucose enrichments were determined from total blood after deproteinization by a Zn(OH)2 precipitate as described (23). Briefly, an aliquot of the supernatant was evaporated to dryness to determine the radioactivity corresponding to D-3-3H. In a second aliquot of the same supernatant, glucose concentration was assessed by the glucose oxidase method.
Insulin sensitivity test

Mice were fasted for 6 h and then anesthetized with a ketamine/xylazine ip injection [1 and 0.1% (wt/vol), respectively]. A single iv insulin injection was performed (5 mU/g diluted in PBS). The same volume of PBS was injected in control mice. Ten minutes after insulin injection, mice were killed by cervical dislocation, vast-lateralis muscles rapidly removed, immediately frozen in liquid nitrogen, and stored at −80°C.

Immunoblotting analyses

Frozen tissues were mechanically homogenized in ice-cold buffer [62.5 mM Tris-HCl (pH 6.8) at 25°C, 2% (wt/vol) sodium dodecyl sulfate, 10% glycerol, 50 mM dithiothreitol], including 2 mM orthovanadate (Sigma-Aldrich, Meylan, France), 1 mM phenylmethylsulfonyl fluoride (Sigma-Aldrich), and a cocktail of protease inhibitors (complete EDTA-free; Roche, Basel, Switzerland). Total protein content was measured using BCA protein assay kit (Interchim, Montluçon, France). Samples containing 20 μg total proteins were resolved by 10% SDS-PAGE and transferred to 0.2 μm nitrocellulose membrane (PerkinElmer). Then relevant proteins were revealed using enhanced chemiluminescence detection (ECL Western blot analysis system; GE Healthcare, Amersham, Buckinghamshire, UK). The expression of IL-6, TNF-α, IL-1β, plasminogen activator inhibitor 1, (PAI-1) and F4/80 mRNAs was evaluated by quantitative RT-PCR analysis. Total mRNAs from the liver and both sc (inguinal) and visceral (mesenteric) white adipose tissues were extracted using TriPure reagent (Roche). PCRs were performed using an AbiPrism 7900 sequence detection system instrument and software (Applied Biosystems, Foster City, CA), as described (3). The expression of cytokines and PAI-1 mRNAs was normalized to RNA loading for each sample using 18S rRNA as an internal standard. Primer sequences for the targeted mouse genes are available upon request.

Immunohistochemistry

The mean relative proportion of adipocytes was estimated by a point-counting technique on hematoxylin/eosin counterstained sections of paraffin-embedded adipose tissues. The number of adipocytes per microscopical field (density) was determined at a magnification of ×250. Macrophages were identified by CD68 immunostaining (1:500; rat antimouse CD68 monoclonal antibody, ABCYS). The number of CD68-positive cells per microscopical field was determined and divided by the total number of adipocytes.

Biochemical analyses

Insulin concentrations were determined in fasted (6 h) state using an ELISA kit (Mercodia, Uppsala, Sweden). Plasma free fatty acid (FFA) and triglyceride concentrations were determined using the COBA COBAS-MIRA analyzer (ABBott Diagnostics, Northampton, UK). Liver triglycerides were determined as described by Cani et al. (3) using a commercial kit (triglycerides enzymatic PAP 150; Biomerieux, Marcy l’Etoile, France). Plasma adiponectin and cytokines concentrations were determined using Multiplex Immunoasay Technology (MILLIPLEX mouse adiponectin and cytokines concentrations were determined using Multiplex Immunoassay Technology Xmap (MILLIPLEX mouse adiponectin and mouse cytokine/chemokine panel, 4 Plex; Millipore, St Quentin en Yveline, France).

Statistical analysis

Results are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism version 4.00 for windows (GraphPad Software, San Diego, CA; www.graphpad.com). To analyze the results from glucose tolerance tests, ANOVA tests were performed on area under the curve (AUC) data. Between-group differences were analyzed using the Student’s t test. P < 0.05 was considered statistically significant.

Results

Estradiol administration protects from the glucose intolerance induced by high-fat feeding mice

We first determined whether chronic E2 administration regulates glucose tolerance in HFD-fed mice. To this end, 4-wk-old mice were divided into two groups: E2-treated (n = 9) and control (n = 9). E2 was administered subcutaneously twice per week (100 μg/kg body weight) for 10 weeks. Blood glucose levels were measured before and 120 min after insulin injection (0.5 U/kg body weight). The area under the curve (AUC) was calculated and compared between groups. The results showed that E2 administration significantly reduced blood glucose levels in response to insulin injection, indicating improved insulin sensitivity (Figure 1).

FIG. 1. E2 administration protects from glucose intolerance and insulin resistance in HFD-fed ovariectomized mice. IPGTT (1 g/kg) in mice fed for 1 or 3 months [A, ovariectomized mice (Ovx), HFD, closed squares] and ovariectomized mice treated with E2 (Ovx+E2, HFD, closed triangles) fed with a HFD; ovariectomized mice (Ovx, NCD, open squares) and ovariectomized mice treated with E2 (Ovx+E2, NCD, open triangles) were fed with a NCD. The inset represents the AUC for each group. Twenty mice per group are considered. Data are means ± SEM. ANOVA statistical analysis of AUC data: *P < 0.05 for Ovx+E2, HFD vs. Ovx, HFD mice; **P < 0.01 for Ovx+E2 vs. Ovx, NCD mice. C, in vivo glucose use rate in sc adipose tissue (SC), ovariatic adipose tissue (Ov), brown adipose tissue (BAT), heart, skin, vastus lateralis muscle (VL), extensor digitorum longus muscle (EDL), and soleus muscle (Sol) in Ovx (n = 6) and Ovx+E2 (n = 6) mice in euglycemic hyperinsulinemic conditions. Data are means ± SEM. *P < 0.05; **P < 0.01 vs. Ovx mice.

To further investigate the mechanisms underlying the protective effect of E2 on glucose metabolism, we analyzed liver triglyceride levels. As shown in Figure 2, E2 administration significantly reduced liver triglyceride levels in HFD-fed mice (n = 6) compared to control mice (n = 6) (P < 0.01). These findings suggest that E2 administration improves glucose tolerance and reduces liver triglyceride levels in HFD-fed mice.
investigated whether E2 administration influences the impair-
ment of insulin sensitivity induced by the HFD. As demonstrated by the euglycemic hyperinsul cinemic clamp analyses performed in placebo-treated mice, our experimental conditions led to a 20 and 50% decrease in insulin action, respectively, after 1 or 3 months of high-fat feeding (Fig. 1B). Of note, E2 adminis-
tration significantly protected ovarioctomized mice from HFD-induced insulin resistance. Indeed, in E2-treated ovarioctomized mice, insulin action was totally preserved after 1 month of high-fat feeding and was reduced by only 25% after 3 months (Fig. 1B).

Glucose use rates during the clamp procedures were not affected by E2 administration in NCD-fed mice, suggesting that the hormone selectively exerts its metabolic effect in the high-fat feeding condition (Fig. 1B). Accordingly, in contrast to results obtained in mice fed with the NCD (Fig. 1C), tissue-specific glucose uptake measured at the end of the euglycemic hyperinsulinemic clamps in mice fed with the HFD for 1 month indicated that the insulin-stimulated glucose use index was significantly increased by E2 administration for the sc and ovarian adipose tissues, brown adipose tissue, and the vast lateralis muscle (Fig. 1C). A nonsignificant similar trend was observed for the soleus muscle (Fig. 1C). As expected, glucose use rate was significantly lower in HFD-fed mice than NCD control mice for most of studied tissues (sc and ovarian adipose tissues, brown adipose tissue, heart, vastus lateralis, and extensor digitorum longus muscles). Altogether these results indicate that E2 administra-
tion reduced the occurrence of HFD-induced insulin resistance.

Interestingly, body weight and fasting plasma insulin, as an index of insulin resis-
tance, were significantly reduced by E2 treatment in mice fed with the HFD for 3 months (Table 1). However, E2 administration did not influence these parameters after 1 month of high-fat feeding, although the hormonal treatment was shown to totally preserve insulin sensitivity in these short-
term experimental conditions (Fig. 1B). FFA and triglyceride plasma concentra-
tions as well as triglycerides hepatic content were not altered by E2. In contrast, circulating adiponectin levels were signif-

E2 administration prevents insulin resistance in HFD-fed mice

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TABLE 1. Metabolic characteristics of HFD-fed ovarioctomized mice according to E2 administration

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>1 month HFD</th>
<th>3 months HFD</th>
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<tbody>
<tr>
<td></td>
<td>Ovx</td>
<td>Ovx+E2</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>21.4 ± 0.26</td>
<td>20.9 ± 0.31</td>
</tr>
<tr>
<td>Fasting insulin (pg/ml)</td>
<td>7.58 ± 0.63</td>
<td>6.78 ± 0.26</td>
</tr>
<tr>
<td>FFAs (mmol/liter)</td>
<td>1.28 ± 0.11</td>
<td>1.14 ± 0.11</td>
</tr>
<tr>
<td>Hepatic TG (mg/g liver)</td>
<td>8.64 ± 0.87</td>
<td>10.97 ± 0.38</td>
</tr>
<tr>
<td>TGs (mmol/liter)</td>
<td>0.53 ± 0.04</td>
<td>0.65 ± 0.04</td>
</tr>
<tr>
<td>Adiponectin (mg/ml)</td>
<td>17.76 ± 0.82</td>
<td>8.70 ± 1.05*</td>
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Data are mean ± SEM. Body weight (grams), fasting plasma insulin (picograms per milliliter), FFAs (millimoles per liter), triglycerides (TG; millimoles per liter), and adiponectin (milligrams per milliliter) in placebo- (Ovx, n = 6) and E2-treated ovarioctomized mice (Ovx+E2, n = 6) fed with the HFD for 1 and 3 months. Hepatic TG (milligrams per gram of tissue) were also quantified in all groups. Ovx, Ovariectomized.

* P < 0.05 vs. Ovx mice, according to the t test statistical analysis.

ovoctomized female mice were treated with placebo or E2 and were concomitantly fed with the HFD for 1 or 3 months. As soon as 1 month later, placebo-treated ovarioctomized mice were characterized by glucose intolerance compared with NCD-fed mice (Fig. 1A). In addition, a significant increase in fasting glycemia was observed in placebo-treated mice fed with the HFD for 3 months (Fig. 1A). Demonstrating the beneficial effect of E2 administration, glucose tolerance was significantly improved in E2-treated mice exposed to the HFD for 1 or 3 months (P < 0.05 and P < 0.001, respectively, according to ANOVA statistical test performed on AUC results) (Fig. 1A). Furthermore, exposure to E2 totally prevented the fasting hyperglycemia induced by 3 months of high-fat feeding in ovarioctomized mice. Interestingly, in NCD-fed ovarioctomized mice, E2 administration did not influence fasting glycemia and resulted in only a weak reduction of glycemic levels during the glucose tolerance tests (Fig. 1A). These data demonstrate that E2 administration reduced the impact of HFD-induced hyperglycemia.

FIG. 2. E2 improves insulin (Ins) signaling and AMPKα activation in skeletal muscles. Representative Western blot analysis of intracellular insulin effectors (A) and AMPKα phosphorylation (B) in vast-lateralis muscles from HFD-fed ovarioctomized (Ovx) and ovarioctomized mice treated with E2 (Ovx+E2) under basal or in vivo insulin-stimulated conditions. Results are means ± SEM (n = 3/group). **, P < 0.01; ***, P < 0.001 vs. Ovx mice. GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.
significantly reduced in E2-treated mice exposed to the HFD for either 1 or 3 months (P < 0.001) (Table 1).

E2 administration improves insulin signaling in muscles

Then, to address the mechanism of the beneficial effect of E2, we investigated whether the hormone improves insulin signaling in peripheral tissues. As shown in skeletal muscles (vast-lateralis) under in vivo insulin-stimulated conditions, E2 administration enhanced IRS-1 protein concentration (1.7-fold, P < 0.01) (Fig. 2A). Furthermore, the concentrations of the phosphorylated form of Akt at the ser 473 (1.6-fold, P < 0.01) and the thr 308 (2.7-fold, P < 0.01) sites were significantly increased in muscles from E2-treated mice, demonstrating that E2 preserves insulin signaling from the deleterious effect of HFD feeding (Fig. 2A). Finally, E2 treatment increased the concentration of AMPK phosphorylation form, only in the presence of insulin, in skeletal muscles (Fig. 2B).

E2 administration modulates the expression of inflammatory factors in HFD-fed mice

Because estrogens are known to modulate cytokine gene expression in numerous pathophysiological animal models (18), we aimed to determine whether E2 administration to ovariectomized mice influences the early inflammatory response induced by high-fat feeding. To this end, we first determined the plasma concentrations of major inflammatory cytokines. None of them were detected in the plasma from both placebo- and E2-treated mice fed with the NCD (data not shown). In contrast, although E2 treatment did not influence the level of cytokines after 1 month of high-fat feeding, it significantly increased the plasma levels of IL-6 (16.6-fold, P < 0.001), TNF-α (16.5-fold, P < 0.05), and IL-1β (2.8-fold, P < 0.05) in mice fed with this diet for 3 months (Fig. 3A). To further characterize the early inflammatory response induced by high-fat feeding, the abundance of the mRNA coding for major cytokines and for PAI-1 was then determined in the liver and adipose tissues, as previously described (3). As shown in Fig. 3B, during the short-term high-fat feeding period, E2 treatment significantly increased the level of IL-6, TNF-α, and PAI-1 mRNAs in both the liver and visceral adipose tissue, compared with results obtained in placebo-treated ovariectomized mice. Furthermore, although E2 did not alter cytokine mRNA levels in sc adipose tissue (data not shown), a significant increased in the number of CD68-positive cells was identified at this site in E2-treated mice, with a similar trend in visceral adipose tissue (Fig. 3C). In addition, E2 administration resulted in a significant elevation of F4/80 mRNA levels in sc (2.1-fold, P < 0.05) and visceral (1.6-fold, P < 0.01) adipose tissues, providing further evidence that E2 increases the density of macrophages in adipose tissues (Fig. 3C).

Both metabolic and inflammatory effects of E2 are mediated by ERα

To determine whether ERα selectively mediates these effects of E2, ERα−/− female mice were ovariectomized, treated or not with E2, and concomitantly fed with the HFD for 1 month. As expected, whereas body weight and fasting plasma insulin concentrations were not significantly altered, E2 treatment significantly improved glucose tolerance, enhanced the expression of inflammatory factors in the visceral adipose tissue (Fig. 4A) as well as in the liver (data not shown), and increased F4/80 mRNA levels in sc and visceral adipose tissues (data not shown) in ERα+/− control mice. In contrast, both proinflammatory and metabolic effects were totally abolished in ERα−/− mice, extending the crucial role of this nuclear receptor to the present HFD model.

ERα deficiency in bone marrow-derived cells alters the proinflammatory but not the metabolic effect of estradiol

The unexpected observation that, although beneficial on insulin sensitivity and glucose tolerance, E2 administration exac-
erbates the inflammatory response induced by high-fat feeding in the liver and visceral adipose tissue prompted us to hypothesize that the respective effects of E2 on inflammation and glucose metabolism are not related, and could involve distinct cellular targets. To address this question, we generated chimeric mice by grafting bone marrow cells from either wild-type or ERα−/− mice in lethally irradiated wild-type ovariectomized female mice, as previously described (24). ERα gene invalidation was individually assessed in peripheral blood mononuclear cells, peritoneal macrophages, and splenocytes by PCR analyses (data not shown). These mice were submitted to the HFD, with the concomitant administration of either placebo or E2 for 1 month. As expected, E2 treatment significantly enhanced the expression of cytokine mRNA expression in the liver and visceral adipose tissue (data not shown).

Interestingly, ERα deficiency in immune cells did not alter the specific glucose use was enhanced in sham-operated HFD-fed mice for the sc and ovarian adipose tissues and most of the muscle sites examined (Fig. 5B). Furthermore, in accordance with data obtained with E2 administration, endogenous estrogens were associated with an increased cytokine mRNA expression in the liver and visceral adipose tissue (data not shown).

Glucose tolerance was also significantly improved in sham-operated mice exposed to the HFD for 1 or 3 months (Fig. 5C). Furthermore, endogenous estrogens prevented the fasted hyperglycemia induced by 3 months of high-fat feeding in ovariectomized mice. Interestingly, in NCD-fed mice, neither ovariectomy nor glycemic influenced the fasting glycemia level during the glucose tolerance tests (Fig. 5C). Altogether these results indicate that endogenous estrogens reduce the occurrence of HFD-induced insulin resistance and glucose intolerance.

**Discussion**

Our data clearly demonstrate that a chronic *in vivo* exposure to estrogens reduced the occurrence of HFD-induced insulin resistance in the mouse, leading to an improved adipose tissue and muscle glucose use rate and insulin signaling. Furthermore, we show that this beneficial metabolic effect occurs despite an increased inflammatory tone.
contribution to glucose homeostasis in a normal metabolic state, estrogens reveal their early and strong preventive or protective effect when the animals are exposed to a diabetogenic stress such as a HFD.

The second main finding of our study is that estrogens, especially chronic E2 administration to ovariectomized mice, enhance the expression of inflammatory factors induced by a HFD in tissues directly involved in insulin action and glucose metabolism, finally leading to the elevation of plasma cytokine concentrations. Furthermore, our observations in chimeric mice strongly suggest that the proinflammatory effect of E2, at least in visceral adipose tissues, results from the ERα-dependent activation of bone marrow-derived cells. These data fit nicely with the conclusion from recent animal studies reporting the proinflammatory influence of estrogens in vivo. Indeed, despite some in vitro studies suggesting that estrogens could exert antiinflammatory properties on several immune cells, in vivo E2 administration was clearly demonstrated to enhance the production of numerous proinflammatory cytokines by conventional antigen-specific CD4+ T lymphocytes, and natural killer T cells, as well as antigen-presenting cells (24, 29, 30).

Interestingly, in HFD-fed mice, the early inflammatory response was recently shown to result from an increase in plasma lipopolysaccharide concentrations, which lead to the activation of CD14-Toll-like receptor 4-expressing cellular targets (3, 31). Among them, macrophages are known to infiltrate both the liver and adipose tissues and thus to largely contribute to the secretion of inflammatory mediators (19). Interestingly, in our model, macrophage density in adipose tissues was significantly increased by E2 treatment. Thus, it is tempting to speculate that a direct effect of estrogens on these pivotal cells of the innate immune system could explain the increase in cytokine expression induced by both endogenous estrogens and E2 substitution in ovariectomized mice. Supporting this hypothesis, monocytes/macrophages, as lymphocytes and dendritic cells, express ERs, especially ERα (18). Furthermore, contrasting with previous in vitro studies suggesting that E2 exerts antiinflammatory properties on lipopolysaccharide-activated monocyte/macrophage cell lines, we recently demonstrated that in vivo E2 administration increases the capacity of murine peritoneal macrophages to produce proinflammatory cytokines (IL-1, IL-6, and IL-12) on Toll-like receptor 4 stimulation (32), as previously reported in microglial cells, the resident macrophages of the brain (30). This proinflammatory effect of in vivo exposure to estrogens is mediated through ERα activation and results from an increase in the activity of the nuclear factor-κB pathway (32).

To our knowledge, the present study was the first to investigate the therapeutic effect of exogenous estrogens on diabetes, insulin resistance, and glucose intolerance in NCD- and HFD-fed wild-type mice. To date, all the animal studies that examined the influence of estrogens on glucose metabolism have been conducted in monkeys, rats, or transgenic mice fed with a normal chow or a moderately fat-enriched diet (10, 11, 25–27). Accordingly to these previous works, we demonstrate here that the beneficial effect of estrogens on insulin sensitivity and glucose tolerance in HFD-fed mice are mediated through ERα activation (15, 17). Conversely, ERβ appeared dispensable because ERβ-deficient mice demonstrated a normal glucose tolerance (28), although no complete metabolic study has been reported to date in this model. Interestingly, although our results indicate that the activation of the estrogen pathway prevents the occurrence and the course of insulin resistance and glucose intolerance resulting from high-fat feeding in wild-type mice, we did not observe any effect of either endogenous estrogens or chronic E2 administration on insulin sensitivity, and only a weak effect of E2 treatment on glucose tolerance, in our normal chow-diet experimental condition. This latter observation suggests that, despite a modest
However, because the low-grade inflammatory tone induced by high-fat feeding initiates insulin resistance and glucose intolerance (2, 3) and because inflammatory cytokines are known to impair insulin signaling, the proinflammatory effect of estrogens should be considered as controversial. However, bone marrow graft experiments indicated that the main part of this proinflammatory effect results from a direct hormonal role on hematopoietic cells. This allows us to conclude that the beneficial metabolic influence of estrogens cannot be explained by the modulation of cytokine expression but results from a direct targeting of nonhematopoietic cells that overcome the deleterious effect of HFD-induced inflammation. In this line, despite their proinflammatory potential, estrogens have been demonstrated to exert protective effects in several inflammatory disease models, preventing target organs or tissues (24, 33). Thus, the inflammatory potential of the actors of the inflammatory-immune system can be balanced by the action of E2 on extramedullar cell targets, which should be now investigated.

Although ERα signaling has been demonstrated to regulate weight gain and adiposity, as confirmed by our observations in mice fed with the HFD for 3 months, we found that, after 1 month of high-fat feeding, E2 administration improved insulin sensitivity and glucose tolerance without any significant effect on body weight. Alternatively, previous studies reported that ERα signaling improves insulin sensitivity through the modulation of lipogenic genes and signal transducer and activator of transcription 3 in the liver (26, 28) and through the regulation of IRS-1 phosphorylation or intra-cellular extracellular resistin distribution in 3T3-L1 adipocytes (34, 35), but the cellular and molecular mechanisms underlying the effect of estrogens on insulin action remain poorly understood. In the present study, E2 administration did not influence plasma triglyceride and FFA concentrations as well as triglyceride content and phosphoenolpyruvate carboxykinase mRNA abundance in the liver (data not shown), and circulating adiponectin levels were significantly decreased in E2-treated mice, as recently reported (27). Furthermore, as described above, our experiments ruled out the hypothesis that estrogens could exert their beneficial metabolic effect through the downmodulation of the inflammatory response to high-fat feeding. In contrast, the demonstration that E2 enhances IRS-1 expression and Akt phosphorylation in insulin-stimulated muscles suggests that the estrogens pathway could directly interfere with insulin signaling in peripheral insulin-sensitive tissues. However, the mechanisms leading to the regulation of IRS-1 expression remains to be clarified because the effect of E2 was observed only after insulin stimulation. Interestingly, AMPKα phosphorylation in muscles was also increased by E2 treatment, suggesting that alternative pathways could be responsible for the improved metabolic status.

These data are in line with a recent study showing a significant improvement in insulin sensitivity and glucose tolerance in male mice submitted to a soy-enriched diet, which were also characterized by an increase in the activation of the AMPKα in skeletal muscles (36). Importantly, because E2 administration enhanced AMPKα phosphorylation in muscles from HFD-fed mice in the present work, although under in vivo insulin stimulation, the involvement of this specific pathway in the beneficial metabolic effect of estrogens should be further investigated. Finally, the role of ERα activation in the central nervous system could play a crucial role in the metabolic effect of these hormones (37). Indeed, using RNA interference mediated by adenovirus-associated viral vectors, Musatov et al. (37) demonstrated that the silencing of ERα in the ventromedial nucleus of the hypothalamus neurons in rodents leads to a phenotype characteristic of the metabolic syndrome, including impaired glucose tolerance.

In conclusion, the present study demonstrates that, in addition to lifestyle modifications, the activation of the estrogen pathway could represent an effective strategy for the prevention of type 2 diabetes, especially in postmenopausal women. However, randomized interventional studies clearly indicated that, besides their metabolic beneficial action, estrogens-based therapy induces several deleterious effects such as an increase in breast cancer and thromboembolic disease incidences, thus leading to an uncertain benefit to risk ratio at the individual level (33). Therefore, based on our knowledge of selective ER modulators, it is now crucial to develop new compounds able to avoid the deleterious effects and maintain the beneficial action of ERα activation on glucose metabolism.

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