Central versus peripheral impact of estradiol on the impaired glucose metabolism in ovariectomized mice on a high-fat diet

Rika Yonezawa,1* Tsutomu Wada,2* Natsumi Matsumoto,2 Mayuko Morita,2 Kanae Sawakawa,2 Yoko Ishii,3 Masakiyo Sasahara,3 Hiroshi Tsuneki,1 Shigeru Saito,1 and Toshiyasu Sasaoka2

1Department of Obstetrics and Gynecology, University of Toyama, Toyama, Japan; 2Department of Clinical Pharmacology, University of Toyama, Toyama, Japan; and 3Department of Pathology, University of Toyama, Toyama, Japan

Submitted 13 December 2011; accepted in final form 29 April 2012

Yonezawa R, Wada T, Matsumoto N, Morita M, Sawakawa K, Ishii Y, Sasahara M, Tsuneki H, Saito S, Sasaoka T. Central versus peripheral impact of estradiol on the impaired glucose metabolism in ovariectomized mice on a high-fat diet. Am J Physiol Endocrinol Metab 303: E445–E456, 2012. First published May 1, 2012; doi:10.1152/ajpendo.00638.2011.—Age-related loss of ovarian function promotes adiposity and insulin resistance in women. Estrogen (E2) directly enhances insulin sensitivity and suppresses lipogenesis in peripheral tissues. Recently, the central actions of E2 in the regulation of energy homeostasis are becoming clearer; however, the functional relevance and degree of contribution of the central vs. peripheral actions of E2 are currently unknown. Therefore, we prepared and analyzed four groups of mice. 1) Control: sham-operated mice fed a regular diet, 2) OVX-HF: ovariectomized (OVX) mice fed a 60% high-fat diet (HF), 3) E2-SC: OVX-HF mice subcutaneously treated with E2, and 4) E2-ICV: OVX-HF mice treated with E2 intracerebroventricularly. OVX-HF mice showed increased body weight with both visceral and subcutaneous fat volume enlargement, glucose intolerance, and insulin resistance. Both E2-SC and E2-ICV equally ameliorated these abnormalities. Although the size of adipocytes and number of CD11c-positive macrophages in perigonadal fat in OVX-HF were reduced by both E2 treatments, peripherally administered E2 decreased the expression of TNFα, lipoprotein lipase, and fatty acid synthase in the white adipose tissue (WAT) of OVX-HF. In contrast, centrally administered E2 increased hormone-sensitive lipase in WAT, decreased the hepatic expression of gluconeogenic enzymes, and elevated core body temperature and energy expenditure with marked upregulation of uncoupling proteins in the brown adipose tissue. These results suggest that central and peripheral actions of E2 regulate insulin sensitivity and glucose metabolism via different mechanisms, and their coordinated effects may be important to prevent the development of obesity and insulin resistance in postmenopausal women.

Increasing in obesity and metabolic syndrome (MetS) are becoming a problem worldwide. In women, body fat composition changes from subcutaneous fat deposition to visceral obesity postmenopause, resulting in an increased incidence of MetS (13, 39). The estrogen deficiency in a postmenopausal state is a crucial factor affecting these metabolic abnormalities (7, 39).

Estrogen is one of the most important hormones, regulating sexual function and reproduction (19, 23). Estrogen is also known to be multifunctional, with roles in the regulation of human physiology, including glucose and lipid metabolism, the maintenance of bone turnover, and neurological functions, especially in women (19, 23, 36). In particular, its involvement in insulin sensitivity and glucose homeostasis is clinically important. An elevation of estrogen levels during late pregnancy is thought to be involved in the induction of maternal insulin resistance (33). In contrast, decreased estrogen levels postmenopause are related to the development of visceral fat obesity and onset of type 2 diabetes (2, 7, 23). In an animal model, an estrogen deficiency in aromatase knockout mice caused increased adiposity and insulin resistance (21, 24). However, the mechanisms underlying the regulation of energy and glucose homeostasis by estrogen are not fully understood.

Estrogen has been thought to exert its biological functions through classical genomic actions initiated by direct binding to the estrogen receptors (ER) (19, 23). Previous studies utilizing ERα and ERβ knockout mice showed a predominant role for ERα in the regulation of glucose metabolism (3, 20). In 3T3-L1 adipocytes, we have previously reported that a low dose of estrogen enhanced insulin signaling at insulin receptor substrate 1 through ERα (27). In addition, estrogen stimulated Akt and AMP kinase in rat soleus muscle, and enhanced glucose uptake via ER by facilitating glucose transporter 4 (GLUT4)’s translocation to the plasma membrane in C2C12 myotubes (12, 32). On the basis of these studies in vitro, the beneficial effects of estrogen on glucose and lipid metabolism appear to be exerted via a direct action in peripheral tissues.

On the other hand, earlier studies indicated that estrogen has an impact on food intake and physical activity (6). Recent studies further identified the central actions of estrogen implicated in the regulation of energy homeostasis and leptin sensitivity (8, 26). Like leptin, estrogen induces the phosphorylation of STAT3 and an increase in the number of excitatory inputs to proopiomelanocortin (POMC) neurons in the arcuate nucleus of the mouse hypothalamus (17). On the basis of these reports, the influence of estrogen on glucose metabolism, particularly its central action, has been examined intensively. However, functional relevance of the central and peripheral actions of E2 and degree of their contribution are currently unknown. Most studies have demonstrated that the administration of estrogen improves glucose metabolism, but the mechanism is explained by various factors, such as decreased food intake, enhanced energy expenditure, and/or reduction of fat volume. In addition, there are potential limitations in differentiating the central actions of estrogen from the peripheral actions, since estrogen was administered peripherally. Moreover, the definition of the central actions of estrogen seems to be obscured by the use of different dosages in the peripheral administration among studies.

Address for reprint requests and other correspondence: T. Sasaoka, Dept. of Clinical Pharmacology, Univ. of Toyama, 2630 Sugitani, Toyama, 930-0194, Japan (e-mail: tsaasaoka@pha.u-toyama.ac.jp).

* Rika Yonezawa and Tsutomu Wada contributed equally to this article.

http://www.ajpendo.org 0193-1849/12 Copyright © 2012 the American Physiological Society E445
On the basis of the previous findings, we hypothesized that the central and peripheral actions of estrogen contribute to the maintenance of glucose and energy metabolisms, possibly to different extents and/or by distinct mechanisms. In the present study, to perform a more appropriate comparison of the central vs. peripheral ameliorating effects of estrogen on energy and glucose homeostasis, we prepared ovariectomized mice fed a high-fat diet as an animal model of postmenopausal obesity, and administered estradiol (E2) via the intracerebroventricular or subcutaneous route. We found that the central and peripheral administration of E2 almost equally prevented the development of glucose intolerance and adiposity, but it independently affected gene expression profiles in peripheral tissues, including white adipose tissue (WAT), brown adipose tissue (BAT) and liver. Thus, this study provides a novel insight into the coordinated and/or complementary mechanisms behind the central and peripheral actions of estrogen to prevent the development of obesity and insulin resistance in postmenopausal women.

MATERIALS AND METHODS

Animals and experimental design. Eight-week-old female C57BL6/J mice purchased from Japan SLC (Shizuoka, Japan) were either ovariectomized (OVX) or sham-operated. The mice were maintained under conditions with standard light (12:12-h light-dark cycle), temperature (24 ± 1°C), and humidity (55 ± 10%), and fed a normal diet composed of 4.8% fat (CE-12; Clea Japan, Tokyo, Japan) and provided water ad libitum. At the age of 10 wk, the normal Diet (ND) was changed to a high-fat diet (HF; 60% fat diet, D12492; Research diets, New Brunswick, NJ). At the age of 14 wk, the mice were anesthetized with pentobarbital sodium (5 mg/kg), and an intracerebroventricular cannula with an osmotic pump was implanted into the left lateral ventricle (0.3 mm posterior to the bregma, 0.9 mm lateral from the central structure, and 2.5 mm below the skull). Artificial cerebrospinal fluid (ACSF, Tocris Bioscience, Bristol, UK) was continuously injected by an osmotic pump (Alzet Brain Infusion Kits3; Alzet Osmotic pump model 1002 or 1004; DURECT, Cupertino, CA) subcutaneously placed on the back (40).

After a 2-wk recovery period (16 wk of age), the mice were divided into three groups; OVX-HF: control ACSF was continuously administered in the absence of E2; E2-SC: water soluble E2 was subcutaneously administered (Sigma-Aldrich, St. Louis, MO; E2 dose; 50 μg·kg⁻¹·day⁻¹) by implanting another pump on the other side of the back; and E2-ICV: for the intracerebroventricular administration of E2, the osmotic pump containing ACSF was replaced with one containing E2 (1 μg·kg⁻¹·day⁻¹). For the experimental controls, sham-operated mice (control), ovariectomized mice fed ND (OVX-ND), and sham-operated mice fed a HF diet for 6 wk (Sham-HF) were prepared. All of the mice except E2-ICV received intracerebroventricular administration of ACSF by the osmotic pump. Characteristics of the mice were analyzed during 10 days after the administration (Fig. 1A). Then, the mice were killed under anesthesia, and tissues were collected. The results of control, OVX-HF, E2-SC, and E2-ICV are presented in the figures, while the results of OVX-ND and sham-HF are described in Table 3 as experimental controls.

In the pilot study, we examined the dose-response relationship of E2 administration. Subcutaneous administration of saline and E2 at 1 μg·kg⁻¹·day⁻¹ (dose for administration in E2-ICV) did not affect glucose metabolism, whereas the E2 at 90 μg·kg⁻¹·day⁻¹ apparently increased the serum concentration and endometrial proliferation beyond normal physiological levels. Intracerebroventricular administration of E2 at 0.01 and 0.1 μg·kg⁻¹·day⁻¹ did not affect glucose metabolism, while the E2 at 1 and 5 μg·kg⁻¹·day⁻¹ almost equally improved the metabolism. In contrast, the elevation of serum E2 level was observed when E2 was intracerebroventricularrly administered at 50 and 500 μg·kg⁻¹·day⁻¹. On the basis of these results, the dosage of E2 used in this study was determined as shown above.

All experimental procedures used in this study were approved by the committee of Animal Experiments at University of Toyama.

Measurements of serum parameters. Mice were deprived of food overnight, and blood samples were collected from the abdominal aorta under anesthesia. After centrifugation at 15,000 g for 5 min, the supernatants of the blood samples were separated and subjected to measurements. Serum estradiol levels were measured with an estradiol enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Serum levels of insulin and leptin were measured using respective ELISA kits (Morinaga, Kanagawa, Japan). Blood glucose levels were measured with a Free Style NIPRO (NIPRO, Osaka, Japan). Serum levels of cholesterol, triglyceride, and HDL cholesterol were determined with a colorimetric kit (Wako Pure Chemical, Osaka, Japan) (41). The serum analyses by ELISA and ELISA kit were conducted in duplicate. The interassay coefficients of variation were less than 10% in each analysis.

Glucose and insulin tolerance tests. Glucose and insulin tolerance tests were conducted as described previously (41). In brief, for the glucose tolerance test (ITT), mice fasted for 16 h were injected intraperitoneally with glucose (2 g/kg body wt) and subsequently injected with human regular insulin (0.75 U/kg body wt) intraperitoneally. Blood samples were collected from a tail vein at 0, 30, 90, and 120 min after the injection.

Analysis of fat mass by MRI imaging. Body fat composition was analyzed by MRI under anesthesia at 10 days after the administration of E2. Series of T1-weighted axial slice were obtained by MRmini SA (DS Pharma Biomedical, Osaka, Japan). The volumes of visceral and subcutaneous adipose tissues from the diaphragm to anus were analyzed with the software Image J (National Institutes of Health, Bethesda, MD). The sum of fat area in each slice × slice thickness was determined.

Histological analysis and immunohistochemistry. Isolated perigonadal adipose and uterus tissue were fixed in 10% formaldehyde for 24 h and embedded in paraffin. Sections 6-μm thick were stained with hematoxylin-and-eosin (HE). The number of adipocytes was counted under low-power fields in each section using a microscope (BX61; Olympus, Tokyo, Japan). The area of adipocytes was traced and measured in 300 cells per mouse by using a digital video analyzer (VH Analyzer VH-H1A5; Keyence, Osaka, Japan). For CD11c immunostaining, the paraffin-embedded sections were incubated with a hamster anti-mouse CD11c antibody (dilution, 1:100, 10 μg/ml) for 3 h followed by a goat anti-hamster IgG antibody (1:100, 8 μg/ml) for 1 h (14).

Real-time quantitative PCR. The RNA extraction, reverse transcription, and real-time PCR using SYBR Green were performed as described previously (41, 42). The relative expression of objective mRNAs was calculated as a ratio to that of S18 ribosomal protein. Primer sequences are listed in Table 1.

Energy consumption, locomotor activity, and core body temperature. O2 consumption (V̇O2), energy consumption, respiratory quotient (RQ), and locomotor activity (counted by inflated ray sensor system) were measured with metabolic chambers (MK-5000RQ, Muromachi Kikai, Tokyo, Japan) at days 5 to 7 after E2 administration with free access to food and water. Rectal temperature was monitored using an electronic thermometer (PTC-301; Unique Medical, Tokyo, Japan) under random-fed conditions.

Statistical analysis. Data are expressed as the means ± SE. P values were determined by one-way ANOVA with post hoc test (Bonferroni’s correction), and P < 0.05 was considered significant.

RESULTS

Influences of E2 administration on serum levels and uterine weights in mice. Serum concentrations of E2 were significantly decreased in OVX-HF (P < 0.01). Serum E2 levels remained reduced in E2-ICV, whereas in E2-SC, they were comparable to

AJP-Endocrinol Metab  •  doi:10.1152/ajpendo.00638.2011  •  www.ajpendo.org
control values (Fig. 1B). Similarly, compared with the control mice, the weight of the uterus (Fig. 1C) and endometrial proliferation (Fig. 1D) was decreased in OVX-HF and E2-ICV, but unchanged in E2-SC. These results indicate that the dose of E2 used in E2-SC matched physiological demands and that the administration of E2 via the intracerebroventricular route did not directly cause any peripheral action under the present experimental conditions.

Table 1. Primers for real-time PCR

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα</td>
<td>AAGCTGTAGCCCACGTCGTA</td>
<td>GGCACCACTAGTTGTCTTGG</td>
</tr>
<tr>
<td>MCP1</td>
<td>CCACTCAAGCTGCTGACTCAT</td>
<td>TGTTGATCTGTTACCTGCCC</td>
</tr>
<tr>
<td>CD11c</td>
<td>ATGTTGGAGGAAGCAAATGG</td>
<td>CCTGGAAATCTCATTGTCCAGA</td>
</tr>
<tr>
<td>FAS</td>
<td>ATCCTGGAGAAGCAAGATGCTTCTT</td>
<td>AGAGAGTTGCTACTCAGGACC</td>
</tr>
<tr>
<td>LPL</td>
<td>AGGCCCATGCTGCTGCTTCTT</td>
<td>GAGAAGGTGAAAAGAGAGAGAG</td>
</tr>
<tr>
<td>HSL</td>
<td>CAGCCGATTGTAAGAAGAGAAGG</td>
<td>TCTTCTGCGACATGCGAGAAG</td>
</tr>
<tr>
<td>PEPCK</td>
<td>CAGGATGGAAGGAAAGGAGATG</td>
<td>AAGCTCAAGTCGAGATCAGAG</td>
</tr>
<tr>
<td>G6Pase</td>
<td>CAAAAGACGCGACGCAAGAGTT</td>
<td>CAGCAAGTGAATCAGGGAG</td>
</tr>
<tr>
<td>UCP1</td>
<td>TACCAAGCTGCGAACATG</td>
<td>AGCGCAAAGTGTGTTCTG</td>
</tr>
<tr>
<td>UCP2</td>
<td>GCCGCGGTCGCGCGCGG</td>
<td>CCGGAGATGGCAGATGAGG</td>
</tr>
<tr>
<td>UCP3</td>
<td>ATGGCAGGGAGAAGGAG</td>
<td>GTGACACCGCTTCTTCTT</td>
</tr>
<tr>
<td>SIP1</td>
<td>ATGTCCAGATGTTGGAGAG</td>
<td>TCACTGCGCTGGCAGTGG</td>
</tr>
</tbody>
</table>

Fig. 1. Influences of peripheral or central administration of E2 on serum E2 levels, uterine weights, and morphology in mice. A: experimental protocol. Mice maintained for 2 wk after ovariectomy (OVX) or sham operation were fed a standard diet or 60% high-fat diet for 4 wk. An intracerebroventricular cannula was continuously injected into the lateral ventricle by an osmotic pump. After 2 wk, continuous subcutaneous administration of E2 (50 μg·kg⁻¹·day⁻¹) was conducted in the E2-SC group of mice, and E2 (1 μg·kg⁻¹·day⁻¹) was intracerebroventricularly injected in the E2-ICV group. Mouse phenotypes were analyzed during 10 days of E2 administration. B: serum E2 levels after 10 days of E2 administration. C: uterine weights after 10 days of E2 administration. Values are expressed as the means ± SE (n = 8–10/group). *P < 0.05, **P < 0.01 compared with control. ††P < 0.01 compared with OVX-HF; §§P < 0.01 compared between E2-SC and E2-ICV, as indicated. D: representative hematoxylin-and-eosin (HE) staining of endometrium. Scale bar = 200 μm.
Characteristics of the ovariectomized mice on a high-fat diet. Body weights of mice before the OVX or sham operation at 8-wk-old were similar among the four groups. After 6 wk on the HFD (16 wk old), body weight was significantly increased in OVX-HF compared with control mice ($P < 0.01$) (Table 2).

Either peripheral (E2-SC) or central (E2-ICV) administration of E2 for 10 days slightly, but significantly, reduced body weights compared with those in OVX-HF (E2-SC: $P < 0.01$, E2-ICV: $P < 0.01$). Fasting blood glucose levels ($P < 0.01$), insulin levels ($P < 0.05$), and leptin levels ($P < 0.01$) were lower in the E2-SC and E2-ICV groups compared with OVX-HF.

Table 2. Metabolic characteristics of mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>OVX-HF</th>
<th>E2-SC</th>
<th>E2-ICV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g, 8-wk-old</td>
<td>18.5 ± 0.4</td>
<td>18.0 ± 0.3</td>
<td>17.4 ± 0.3</td>
<td>17.7 ± 0.2</td>
</tr>
<tr>
<td>Body weight, g, 16-wk-old</td>
<td>22.0 ± 0.3</td>
<td>26.6 ± 0.9**</td>
<td>25.9 ± 1.0**</td>
<td>25.8 ± 0.4**</td>
</tr>
<tr>
<td>Body weight, g, before GTT (fasting)</td>
<td>18.1 ± 0.3</td>
<td>24.8 ± 0.9**</td>
<td>24.5 ± 0.9**</td>
<td>23.3 ± 0.4**</td>
</tr>
<tr>
<td>Body weight, g, before euthanasia</td>
<td>24.1 ± 0.3</td>
<td>27.1 ± 0.6**</td>
<td>25.2 ± 0.5††</td>
<td>24.7 ± 0.4††</td>
</tr>
<tr>
<td>Food intake, kcal/day</td>
<td>15.3 ± 0.9</td>
<td>14.6 ± 0.8</td>
<td>10.0 ± 0.6**,††</td>
<td>13.3 ± 0.5§§</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>95.7 ± 2.8</td>
<td>125.5 ± 10.1**</td>
<td>75.4 ± 4.9**,††</td>
<td>93.4 ± 7.6††</td>
</tr>
<tr>
<td>Fasting insulin, ng/ml</td>
<td>1.75 ± 0.1</td>
<td>2.15 ± 0.2*</td>
<td>2.02 ± 0.2</td>
<td>1.74 ± 0.1†</td>
</tr>
<tr>
<td>Leptin (pg/ml)</td>
<td>0.93 ± 0.3</td>
<td>7.83 ± 1.3**</td>
<td>1.46 ± 0.2††</td>
<td>3.40 ± 0.3**,††§</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>55.0 ± 2.1</td>
<td>68.8 ± 3.4**</td>
<td>69.3 ± 1.3**</td>
<td>73.0 ± 2.5**</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>43.5 ± 1.1</td>
<td>56.8 ± 2.8**</td>
<td>48.9 ± 1.1**,††</td>
<td>58.5 ± 7.5**,§§</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>33.1 ± 1.3</td>
<td>29.4 ± 1.7*</td>
<td>34.6 ± 1.0,††</td>
<td>29.5 ± 1.3§§</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. The control ($n = 11$) group comprised sham-operated mice fed a normal diet. The OVX-HF group ($n = 15$) comprised ovariectomized mice fed a high-fat diet. The E2-SC group comprised OVX-HF mice subcutaneously treated with E2 ($n = 11$), while the E2-ICV group comprised OVX-HF mice treated with E2 intracerebroventricularly ($n = 14$). GTT, glucose tolerance test; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *$P < 0.05$, **$P < 0.01$, compared to control. †$P < 0.05$, ††$P < 0.01$, compared to OVX-HF. §$P < 0.05$, §§$P < 0.01$, compared to E2-SC.

Fig. 2. Effects of peripheral or central administration of E2 on glucose and insulin tolerance tests in ovariectomized mice on a high-fat diet. Four groups of mice, namely control (sham-operated mice fed regular diet), OVX-HF (ovariectomized mice fed high-fat diet), E2-SC (OVX-HF mice subcutaneously treated with E2), and E2-ICV (OVX-HF mice treated with E2 intracerebroventricularly), were prepared. A: glucose tolerance test (GTT; 2 g/kg glucose ip) was performed in overnight-fasted mice. B: insulin tolerance test (ITT; 0.75 units/kg insulin ip) was conducted in random-fed mice. The area under the curve (AUC) over the course of 120 min in each experiment was averaged. Values are expressed as the means ± SE ($n = 10–14$ groups). **$P < 0.01$ compared with control. †$P < 0.05$, ††$P < 0.01$ compared with OVX-HF, as indicated.
Effects of peripheral and central administration of E2 on the development of glucose intolerance and insulin resistance in ovariectomized mice on a high-fat diet. Since fasting glucose and insulin levels were lower in both E2-SC and E2-ICV than in OVX-HF, we further compared glucose metabolism in these mice (Fig. 2). In the GTT, OVX-HF showed greater increases in blood glucose levels at 0, 15, 30, and 60 min than control mice ($P < 0.01$) (Fig. 2A). No increase was observed in E2-SC ($P < 0.01$) or E2-ICV ($P < 0.01$). The area under the curve was equivalent between E2-SC and E2-ICV. It is worth noting that these protective effects of E2 were not associated with abnormal elevations of serum leptin levels ($P < 0.01$), the degree of protection was greater in E2-SC than E2-ICV ($P < 0.05$). Consistent with the estrogen-related increases in serum triglyceride levels that have been reported in the literature (11), there was a significant decrease in OVX-HF, and the administration of E2 via either the SC or intracerebroventricular route certainly elevated serum triglyceride levels compared with those in OVX-HF (Table 2).

Effects of peripheral and central administration of E2 on the body fat composition. Since body weight was reduced in E2-SC ($P < 0.01$) and E2-ICV ($P < 0.01$) compared with that in OVX-HF after 10 days of E2 administration (Table 2), we examined the body fat composition of the mice by MRI (Figs. 3A). Lean mass volumes did not differ among four groups (data not shown). However, visceral and subcutaneous volumes and actual perigonadal fat weights were larger in OVX-HF than control mice, but significantly smaller in E2-SC and E2-ICV than OVX-HF after 10 days of treatment ($P < 0.01$) (Fig. 3, B and C). The degree of change was significantly greater in E2-SC than E2-ICV ($P < 0.01$). No difference in the degree of reduction was observed among visceral, subcutaneous, and perigonadal fat. These results suggest that the reduction of body weight is mostly due to the reduction of body fat in E2-replaced animals.

Influences of central and peripheral administration of E2 on the inflammation and gene expression in adipose tissue and liver. Since the accumulation of body fat was similarly ameliorated by either administration of E2, we next conducted a histological analysis in the perigonadal fat of the mice (Fig. 4). HE staining revealed the size of adipocytes to be markedly increased in OVX-HF compared with the control mice, but smaller in both E2-SC and E2-ICV than OVX-HF (Fig. 4A). Cell-size distribution analyses demonstrated that the adipocyte hypertrophy observed in OVX-HF was markedly prevented by both routes of administration. The average size of adipocytes was significantly smaller in E2-SC than E2-ICV ($P < 0.01$) (Fig. 4, B and C). Similar trends were observed in the inguinal fat of the mice (data not shown). In addition, expression levels of two adipogenic enzymes, lipoprotein lipase (LPL) and fatty acid synthase (FAS), were significantly decreased in perigo-
nadal adipose tissue of E2-SC \((P < 0.01)\), but not E2-ICV, compared with OVX-HF (Fig. 5A). In contrast, the expression of hormone-sensitive lipase (HSL) was significantly elevated only in E2-ICV \((P < 0.01)\) compared with that in OVH-HF (Fig. 5A). Furthermore, the increased mRNA expression of CD11c in OVX-HF was attenuated in E2-SC and E2-ICV (E2-SC: \(P < 0.01\), E2-ICV: \(P < 0.05\)) (Fig. 5B). Similar findings were observed by an immunohistochemical staining with anti-CD11c antibody. Levels of mRNA for monocyte chemoattractant protein 1 (MCP1) and TNF\(_\alpha\) were markedly elevated in OVX-HF (Fig. 5C). Interestingly, the enhanced expression of MCP1 and TNF\(_\alpha\) was suppressed only in E2-SC (MCP1: \(P < 0.01\), TNF\(_\alpha\): \(P < 0.05\)), not in E2-ICV. In the liver, the mRNA expression of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), two rate-limiting enzymes of gluconeogenesis, was suppressed by either route of E2 administration \((P < 0.05)\) compared with that in OVX-HD (Fig. 5D).

**Effects of central and peripheral administration of E2 on energy expenditure.** Recent studies have implicated the hypothalamic action of E2 in the regulation of whole body energy homeostasis and physical activity (17, 26, 29). We next investigated energy balance and locomotor activity in the mice (Fig. 6). \(\dot{V}O_2\) and energy expenditure were significantly decreased in OVX-HF compared with control mice. These reductions were prevented by the central or peripheral administration of E2. These overall changes in energy balance were more apparent in the dark phase than light phase. Interestingly, the RQ was decreased by the high-fat diet, and the level was further decreased only in E2-SC at the dark phase. Spontaneous locomotor activity was reduced in OVX-HF, but significantly greater in E2-ICV \((P < 0.01)\), but not E2-SC, than in OVX-HF. Thus, the decreased energy expenditure in OVX-HF was ameliorated by either route of E2 treatment, although the degree of amelioration was greater in E2-ICV than E2-SC.

**Effects of central and peripheral administration of E2 on core body temperature and uncoupling protein (UCP) expression in BAT.** Since energy expenditure was elevated by the administration of E2, we measured core body temperature in the mice (Fig. 7A). Body temperature did not differ between control mice and OVX-HF. Interestingly, body temperature was significantly elevated in E2-ICV \((P < 0.05\) vs. OVX-HF),
but not in E2-SC. To further clarify the underlying mechanism, the mRNA expression of UCP was examined in BAT. Expression of UCP1 was not altered in OVX-HF but was significantly elevated in E2-ICV compared with OVX-HF (P < 0.01) (Fig. 7B). Similar changes were observed in the expression of UCP2 and UCP3 (Fig. 7B). These results indicate that E2 stimulates thermogenesis by a central regulatory mechanism possibly via the autonomic nervous system.

**DISCUSSION**

The accumulation of visceral fat in postmenopausal women is closely associated with increased morbidity of diabetes, dyslipidemia, and coronary vascular disease (7). Estrogen is considered a major regulator of adiposity and insulin sensitivity, based on clinical evidence supporting hormone replacement therapy in postmenopausal women (18, 35), and studies using ovariectomized animals supplemented with E2 (2, 4, 22, 23, 24, 31). Estrogen also appears to play physiological roles in men, because an apparent phenotype of obesity was seen in male, as well as female, ERα knockout mice (20). A young male with a disruptive mutation of the ERα gene demonstrated impaired glucose tolerance and insulin resistance (37). E2 enhances insulin sensitivity by directly functioning in insulin’s peripheral target tissues (3, 27). In addition, recent studies have shed light on the importance of E2 via its central actions in energy homeostasis and adiposity (17, 26, 29). Although there is accumulating evidence for precise mechanisms of E2 actions, little is known about the relative contribution of central and peripheral actions to the entire metabolic effect of E2 and their functional relationship. In the present study, we prepared OVX-HF as a postmenopausal obese model, because OVX mice fed a normal diet (OVX-ND) and sham-operated mice fed a high-fat diet (Sham-HF) (Table 3 and data not shown) showed only mild glucose intolerance in the GTT and insulin resistance in the ITT without apparent fat accumulation. Ovariectomized mice were fed HFD for 6 wk, since feeding of a high-fat diet to ovariectomized mice for longer periods induced severe obesity, and the influence of HFD feeding rather than...
deficiency of estrogen was dominant compared with our OVX-HF model (22). In the present study, we clearly showed that either central or peripheral administration of E2 similarly improved glucose intolerance and adiposity, although the underlying mechanisms of the improvement appear to be different. The effect of central E2 administration on the metabolism appears to be specific among steroid hormones, since continuous administration of dexamethasone, a synthetic glucocorticoid to the mice deteriorated glucose tolerance in GTT and insulin sensitivity in ITT, and increased food consumption (data not shown), as previously reported (45).

Since systemic E2 transfers to the central nervous system through the blood-brain barrier (29), effects of subcutaneously administered E2 can be exerted via both direct peripheral and central hypothalamic routes. The effect of subcutaneously administered E2 at 50 μg·kg⁻¹·day⁻¹ examined in this study seems to be quite relevant to physiological actions, because serum E2 concentrations and the weight and histological features of the uterus in OVX-HF remained normal with this treatment (Fig. 1). In this regard, the administration via SC at 90 μg·kg⁻¹·day⁻¹ apparently increased the serum concentration and endometrial proliferation beyond normal physiological levels (data not shown). On the other hand, intracerebroventricular administration of E2 at 1, but not 0.1 (data not shown), μg·kg⁻¹·day⁻¹ effectively ameliorated systemic insulin sensitivity (Fig. 2). In addition, E2-ICV at 1 μg·kg⁻¹·day⁻¹ potently increased the expression of HSL and UCPs and locomotor activity in the route-dependent manner. It is noteworthy that the intracerebroventricular administration of E2 at 1 μg·kg⁻¹·day⁻¹ affected neither its serum concentration, glucose metabolism, nor endometrial proliferation in the uterus, whereas the intracerebroventricular administration at 50 μg·kg⁻¹·day⁻¹ increased the peripheral concentration of E2 (data not shown). These results suggest that intracerebroventricularly administered E2 at 1 μg·kg⁻¹·day⁻¹ used is not directly transferred to the peripheral circulation, and the elicited action appears to be mediated by only the central nervous system (CNS). In addition, we consider that intracerebroventricular administration of E2 at 1 μg·kg⁻¹·day⁻¹ provides an appropriate model to estimate the central effect of estrogen.
However, we cannot rule out the possibility that the central concentration of estrogen in E2-ICV might be beyond the range physiologically delivered via the peripheral route, because some of central actions, including the regulation of HSL and UCP expression and locomotor activity in E2-ICV, appear to be greater than those in E2-SC. Further experiments, including the analysis with tissue-specific estrogen receptor knockout mice, might be needed for better understanding of the specific contribution of central vs. peripheral effects of estrogen on the metabolism.

Systemic E2 treatment attenuates lipid uptake and lipogenesis by suppressing the expression of LPL and lipogenic enzymes, including the sterol response element binding protein 1c (SREBP1c)-FAS pathway in WAT (4, 10). In the present study, subcutaneously administered E2, but not intracerebroventricularly administered E2, markedly reduced the expression of LPL and FAS in WAT (Fig. 5A). These results suggest that E2-induced suppression of lipogenesis is mediated via direct action in peripheral adipose tissue. It is also that systemic E2 treatment facilitates lipolysis by upregulation of HSL (9). In contrast to the lipogenesis, expression of HSL was increased in E2-ICV, whereas it tended to be suppressed in E2-SC possibly due to the greater reduction of adipocyte size (10). E2 and leptin are known to transmit signals with similar mechanisms to some extent in the hypothalamus (16, 17). The mechanisms by which the central action of E2 induces the expression of HSL in WAT appear to be similar to those of central leptin actions controlling adipose tissue lipolysis via the autonomic nervous system (5). Along this line, subcutaneously administered E2 rather than intracerebroventricularly administered E2 decreased fat volume and adipocyte size (Figs. 3 and 4), because systemically and centrally administered E2 could
additively influence to the adiposity. These results suggest that systemic E2 directly suppresses lipid uptake and lipogenesis, whereas central E2 enhances lipolysis in WAT. Thus, central and peripheral actions of E2 appear to coordinately regulate adipose tissue volumes in mice (Fig. 7C).

Enhancement of chronic inflammation by accumulation of macrophages in enlarged adipose tissue is implicated in the development of insulin resistance (43). In this regard, expression of MCP1, TNFα, and CD11c, a marker of inflammatory M1 macrophages, was elevated in OVX-HF. Interestingly, subcutaneously administered E2, but not intracerebroventricularly administered E2, suppressed expression of TNFα, whereas the average size of adipocytes, CD11c expression, and immunohistochemical CD11c-positive macrophages were almost equivalent between E2-SC and E2-ICV (Fig. 5). These results indicate that the suppression of TNFα production in adipose tissue appears to be a direct target of E2 as a peripheral effect (Fig. 7C). Also, a recent in vitro study suggests that E2 attenuated TNFα expression by suppressing NF-κB activation via the modification of microRNA expression in primary macrophages (25).

Hepatic function is predominantly affected during the development of systemic insulin resistance in ERα knockout mice (3). Because administration of E2 via both routes suppressed expression of G6Pase and PEPCK in OVX-HF (Fig. 5D), E2 appears to suppress gluconeogenesis via the central action. This finding exhibits similarities to a report that intracerebroventricularly administered leptin suppressed expression of G6Pase and PEPCK in the liver of rats fed a high-fat diet (30).

Regarding the central action of estrogen, it has very recently been reported that mice with genetically disrupted ERα expression in steriodogenic factor-1 neurons at ventromedial hypothalamus (VMH) demonstrated impaired glucose tolerance, reduced energy expenditure, and abdominal obesity, whereas food consumption was unaltered (44). In contrast, POMC-neuron specific ERα knockout mice showed hyperphagia without directly affecting energy expenditure or fat distribution. These results illustrate the functional linkage between POMC neurons and regulation of food intake (1) and between VMH and thermogenesis in BAT (34), and indicate the physiological significance of neuronal regulation of energy homeostasis via ERα. Consistent with these reports, we observed that...
The present findings suggest that CNS-specific E2/ER agonist (5), possibly through activation of the expression of UCP1, UCP2, and UCP3 was also significantly elevated in BAT (Fig. 7B), possibly through activation of the autonomic nervous system similar to leptin (5). These current results clearly indicate that the central action of E2 increases thermogenesis in BAT and locomotor activity, resulting in an increase in whole body energy expenditure.

The administration of E2 to relatively long periods reduced body fat volume and improved glucose metabolism in obese diabetic mice (4, 10, 15, 31). Thus, both the direct effect and the consequence of body fat reduction may be involved in the ameliorative mechanisms of E2. In the present study, however, intracerebroventricularly administered E2 improved glucose intolerance in OVX-HF, despite no apparent changes in body weight at GTT (Table 2, Fig. 2). In addition, since E2 induces leptin secretion, suppressed hepatic glucose production, and increased energy expenditure. The observed changes, including upregulation of HSL in WAT and UCPs in BAT, suppression of G6Pase and PEPlA expression, and chronic inflammation in WAT. Importantly, the effects of centrally administered E2 were almost comparable to those of systemic administration of the impaired glucose metabolism (5, 38). Meanwhile, we found that, in female ovariectomized ob/ob mice, intracerebroventricularly administered E2 injected 15 min before GTT acutely ameliorated the impaired glucose tolerance (data not shown). Therefore, we insist that E2 could acutely modulate the CNS function controlling systemic glucose metabolism without alteration of fat volumes, although future investigation is needed to clarify this issue.

In summary, E2 regulates energy homeostasis and glucose metabolism via the pleiotropic effects mediated by central and peripheral actions. E2, via the central action, stimulated lipolysis, suppressed hepatic glucose production, and increased energy expenditure. The observed changes, including upregulation of HSL in WAT and UCPs in BAT, suppression of G6Pase and PEPlA expression in the liver, and increase of physical activity and thermogenesis are possible mechanisms. On the other hand, E2, via its peripheral action, suppressed lipogenesis, TNFα expression, and chronic inflammation in WAT. Importantly, the effects of centrally administered E2 were almost comparable to those of systemic administration on the impaired glucose metabolism and increased adiposity, without affecting endometrial proliferation in this model of postmenopausal obesity (Fig. 7C, Table 4). In view of health care for women, the present findings suggest that CNS-specific E2/ER agonist might be beneficial to improve obesity and diabetes without affecting E2-dependent malignancies, such as breast and endometrial cancers.

ACKNOWLEDGMENT
We thank Ms. Takako Matsushima (University of Toyama, Japan) for excellent technical assistance.

GRANTS
This work was supported in part by a grant from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to T.W., S.S. and T.S.).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: R.Y., T.W., N.M., M.M., K.S., and Y.I. performed experiments; R.Y., T.W., N.M., M.M., and K.S. analyzed data; R.Y. prepared figures; R.Y. and T.W. drafted manuscript; R.Y., T.W., N.M., M.M., K.S., Y.I., M.S., H.T., S.S., and T.S. approved final version of manuscript; T.W. and T.S. conception and design of research; T.W. interpreted results of experiments; T.W., Y.I., M.S., H.T., S.S., and T.S. edited and revised manuscript.

REFERENCES


