2-Hydroxyestradiol Attenuates the Development of Obesity, the Metabolic Syndrome, and Vascular and Renal Dysfunction in Obese ZSF1 Rats

STEVAN P. TOFOVIC, RAGHVENDRA K. DUBEY, and EDWIN K. JACKSON

Center for Clinical Pharmacology (S.P.T., R.K.D., E.K.J.) and Departments of Pharmacology (E.K.J.) and Medicine (S.P.T., R.K.D., E.K.J.), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; and Department of Obstetrics and Gynecology (R.K.D.), Clinic for Endocrinology, University Hospital, Zurich, Switzerland

Received May 18, 2001; accepted August 30, 2001  This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

A pandemic of obesity is contributing importantly to the prevalence of the metabolic syndrome characterized by hypertension, insulin resistance, and hyperlipidemia. In turn, the metabolic syndrome is contributing to vascular disease and the accelerating epidemic of chronic renal failure. Currently, pharmacological approaches to attenuate obesity and its cardiovascular/renal sequelae are limited. The purpose of this study was to determine the effects of 2-hydroxyestradiol, a metabolite of 17β-estradiol with minimal estrogenic activity, on the development of obesity, the metabolic syndrome, and heart, vascular, and renal dysfunction in obese ZSF1 rats, a well-characterized genetic model of obesity and the metabolic syndrome with concomitant heart, vascular, and kidney disease. ZSF1 rats were treated, beginning at 12 weeks of age, for 26 weeks with vehicle or 2-hydroxyestradiol (10 μg/kg/h). At baseline and after 24 weeks of treatment, animals were placed in metabolic cages, and food intake, water intake, urine output, and urinary excretion of proteins and glucose were determined. Next, in fasting animals, plasma cholesterol was measured, an oral glucose tolerance test was conducted, and total glycated hemoglobin levels were determined. At the end of the study, animals were anesthetized and instrumented for assessment of heart performance, renal hemodynamics, and mesenteric vascular reactivity. 2-Hydroxyestradiol attenuated the development of obesity and improved endothelial function, decreased nephropathy, decreased the severity of diabetes, lowered arterial blood pressure, and reduced plasma cholesterol. 2-Hydroxyestradiol may be an important lead for the development of safe and effective drugs to attenuate obesity and its metabolic, vascular, and renal sequelae.

Obesity is pandemic and worsening in developed countries (Mokdad et al., 2000). Obesity contributes importantly to the metabolic syndrome (Bergman et al., 2001), a disorder characterized by hypertension, insulin resistance, and hyperlipidemia (Reaven, 1994). The metabolic syndrome in turn contributes to heart and vascular disease (Colditz, 1999) and to the accelerating epidemic of end stage renal failure (Hall et al., 1997, 1999). Unfortunately, pharmacological management of obesity has caused, rather than attenuated, cardiovascular disease. For example, phentermine/fenfluramine combination produces valvular heart disease (Lepor et al., 2000) and phenylpropanolamine causes stroke (Kernan et al., 2000). Thus, drugs that attenuate obesity and its metabolic, vascular, and renal sequelae without adversely affecting the heart are badly needed.

Studies by Oparil et al. (1997) suggest that 17β-estradiol causes weight loss. However, it is conceivable that the effects of 17β-estradiol on body weight are mediated in part by its nonestrogenic metabolites, rather than by a direct effect of 17β-estradiol per se. In this regard, our recent studies suggest that several of the cellular effects of 17β-estradiol are mediated by its nonestrogenic metabolites, particularly the catecholestradiols (2-hydroxyestradiol and 4-hydroxyestradiol) and methoxyestradiols (2-methoxyestradiol and 4-hydroxyestradiol) (for reviews, see Dubey and Jackson, 2001a, 2001b). For example, it appears that the ability of 17β-estradiol to inhibit the proliferation of vascular smooth muscle cells and to attenuate the production rate of collagen by vascular smooth muscle cells is mediated, at least in part, by the metabolism of 17β-estradiol to hydroxyestradiols and methoxyestradiols (Dubey et al., 2000; Zacharia et al., 2001). Likewise, proliferation of and collagen synthesis by glomerular mesangial cells (Xiao et al., 2001) and cardiac fibroblasts (Dubey et al., 1998) are inhibited by hydroxyestradiols and methoxyestradiols. Moreover, our studies indicate that hydroxyestradiols and methoxyestradiols inhibit endothelin-1 production by endothelial cells (Dubey et al., 2001). Finally, in vascular smooth muscle cells, catecholestradiols and methoxyestradiols attenuate peroxidation of membrane phospholipids and peroxidation-induced cell growth and migration via nonestrogen receptor-dependent mechanisms (Dubey et al., 1999).

If the effects of 17β-estradiol on body weight are mediated in part by its metabolites, this would provide an important pha-
matic opportunity. Because catecholestradiols and methoxyestradiols exert little estrogenic activity, such compounds may be effective and safe for the attenuation of obesity regardless of gender. Also, because catecholestradiols and methoxyestradiols attenuate growth of vascular smooth muscle cells, cardiac fibroblasts and glomerular mesangial cells, these metabolites of 17β-estradiol may be particularly effective in attenuating the vascular and renal sequelae of obesity. Accordingly, the purpose of this study was to determine the long-term effects of 2-hydroxyestradiol, a major 17β-estradiol metabolite, on body weight, metabolic status, and cardiac, vascular, and renal function in an animal model that is characterized by the spontaneous development of obesity, the metabolic syndrome, and cardiac, vascular, and renal dysfunction.

Materials and Methods

Twenty 12-week-old male obese ZSF1 rats (Genetic Models Inc., Indianapolis, IN) were used. Obese ZSF1 rats were generated by Genetic Models Inc. by crossing lean heterozygous female Zucker diabetic fatty rats (ZDF+/+fa) with lean heterozygous male spontaneously hypertensive heart failure rats (SHHF/Mcc-cp, +/cp; a strain derived from crossing SHR/N rats with Kolletsy rats). Both the fa (Phillips et al., 1996) and cp (Wu-Peng et al., 1997) alleles represent different mutations in the leptin receptor gene, and obese ZSF1 rats have one fa allele and one cp allele. Therefore, ZSF1 rats are compound heterozygotes for the leptin receptor gene locus. As we have described recently (Tofovic et al., 2000), compared with several different rat strains including Wistar-Kyoto normotensive rats, spontaneously hypertensive rats, and obese SHHF/Mcc-cp rats, obese ZSF1 rats have the metabolic syndrome (i.e., hypertension, diabetes, and hyperlipidemia), have left ventricular dysfunction, and develop nephropathy as characterized by massive proteinuria, abnormal renal histopathology (glomerulosclerosis and severe tubulointerstitial and vascular changes), and reduced glomerular filtration rate. Thus, this rat strain develops obesity, the metabolic syndrome, and the end-organ sequelae associated with the metabolic syndrome.

At baseline (12 weeks old and before initiation of treatments) body weight was measured, and animals were placed in metabolic cages for measurement of 24-h food intake, water intake, urine output, and urinary excretion of proteins (bicinchoninic acid method; Pierce, St. Louis, MO). Next, osmotic minipumps (model 2 ML4; Alzet, Rockford, IL) and glucose (Infinity Glucose Reagent; Sigma Diagnostics). After 26 weeks of treatment, animals were fasted overnight, and blood samples (tail vein) for measurement of cholesterol were taken. Plasma samples were analyzed in duplicate for cholesterol levels (Sigma Diagnostics). After 26 weeks of treatment, animals were fasted overnight, an oral glucose tolerance test was conducted, and total glycated hemoglobin levels were determined (Sigma Diagnostics). The oral glucose tolerance test was conducted by obtaining blood samples (tail vein) for plasma glucose before and 2 h after an oral glucose load (2 g/kg by gavage). Plasma glucose levels were measured with the Precision Q.i.d. Blood Glucose Test Strips kit (Medisense, Inc., Bedford, MA).

At the end of 26 weeks of treatment and after the last oral glucose tolerance test, terminal experiments were conducted in anesthetized rats. In this regard, animals were anesthetized and instrumented as described below for assessment of heart performance, renal hemodynamics, and mesenteric vascular reactivity.

For terminal experiments in anesthetized rats (pentobarbital, 45 mg/kg, i.p.), a PE-50 catheter was advanced via the carotid artery into the left ventricle and connected to a heart-performance analyzer (model HPA-210r; Micro-Med, Inc., Louisville, KY) for continuous measurement of 10 time/pressure variables. A PE-50 catheter was inserted into the femoral artery and connected to a blood pressure analyzer (model BPA; Micro-Med, Inc.) for measurement of arterial blood pressure. A PE-10 catheter was inserted into the left ureter for urine collection, and a flow probe (model 1RB; Transonic Systems, Inc., Ithaca, NY) was placed on the left renal artery for determination of renal blood flow. An infusion of 14C-inulin (NEN Life Science Products, Inc., Boston, MA; 0.035 μCi/20 μl saline/min) was initiated, and after 60 min, two 30-min clearance periods were conducted. A mid-point blood sample (300 μl) for measurement of radioactivity was collected. Plasma and urine 14C-inulin radioactivity were measured by liquid scintillation analysis (Tri-Carb, model 2500TR; Packard Instrument Co., Inc., Canberra Industries, Meriden, CT), and renal clearance of 14C-inulin was calculated. A flow probe (model 1RB; Transonic Systems, Inc.) was placed on the mesenteric artery for determination of mesenteric blood flow, and a 32-gauge needle was inserted into the mesenteric artery and attached to a Y-connector for dual intramesenteric artery infusions (25 μl/min each). Angiotensin II (30 ng/min) plus methoxamine (3 μg/min) were delivered via one intramesenteric artery infusion line into the mesenteric vascular bed to preconstrict the mesenteric vascular bed. Next, vascular responses to increasing doses of acetylcholine (0.1, 0.3, and 1.0 μg/min; 5 min per dose) and sodium nitroprusside (0.5, 1.5, and 5.0 μg/min; 5 min per dose) were elicited by injecting these agents via the other intramesenteric artery infusion line into the mesenteric vascular bed. Vascular resistances were calculated as arterial blood pressure divided by blood flow. All values refer to means ± S.E.M. for nine to 10 animals in each group. Statistical analyses (unpaired Student's t test or a repeated measures 2-factor analysis of variance followed by a Fisher's least significance difference test if appropriate) were performed using the Number Cruncher Statistical software program (Kaysville, UT).

Results

As shown in Table 1, at baseline (12 weeks old and before initiation of treatments) control and 2-hydroxyestradiol groups had similar body weights, food and water intakes, urinary volumes, and urinary excretion rates of glucose and protein. This table also shows that 12-week-old ZSF1 rats have polydipsia and polyuria and spill large quantities of glucose and protein into their urine.

As shown in Table 2, after approximately 6 months of treatment and compared with vehicle-treated (control) animals, the 2-hydroxyestradiol-treated animals had significantly lower values for body weight, food intake, urine volume, and urinary excretion rates of glucose and protein. This table also shows that 12-week-old ZSF1 rats have polydipsia and polyuria and spill large quantities of glucose and protein into their urine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values Randomized to Vehicle</th>
<th>Values Randomized to 2-Hydroxyestradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>484 ± 4</td>
<td>483 ± 9</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>41.4 ± 1.6</td>
<td>43.9 ± 1.4</td>
</tr>
<tr>
<td>Water intake (ml/day)</td>
<td>115 ± 10</td>
<td>135 ± 10</td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
<td>94.8 ± 8.2</td>
<td>102 ± 9.2</td>
</tr>
<tr>
<td>Urinary glucose excretion (mg/day)</td>
<td>9.8 ± 0.2</td>
<td>8.5 ± 1.0</td>
</tr>
<tr>
<td>Urinary protein excretion (mg/day)</td>
<td>227 ± 29</td>
<td>258 ± 27</td>
</tr>
</tbody>
</table>
TABLE 2
Values of parameters after 24 to 26 weeks of treatment with either vehicle (control) or 2-hydroxyestradiol in ZDF1 rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>2-Hydroxyestradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>768 ± 14</td>
<td>571 ± 11 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>44.6 ± 1.0</td>
<td>36.6 ± 0.9 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Water intake (ml/day)</td>
<td>131.8 ± 7.0</td>
<td>67.4 ± 4.5 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
<td>86.5 ± 3.0</td>
<td>45.6 ± 3.8 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Urinary glucose excretion (mg/day)</td>
<td>6.5 ± 0.4</td>
<td>1.7 ± 0.5 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Urinary protein excretion (mg/day)</td>
<td>586 ± 41</td>
<td>333 ± 21 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Fasting plasma glucose immediately before oral glucose tolerance test (mg/100 ml)</td>
<td>187 ± 16</td>
<td>148 ± 8 (P &lt; 0.005)</td>
</tr>
<tr>
<td>Plasma glucose 2 h after oral glucose load of 2 g/kg (mg/100 ml)</td>
<td>326 ± 12</td>
<td>265 ± 13 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>12.5 ± 1.7</td>
<td>3.8 ± 0.7 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/100 ml)</td>
<td>399 ± 24</td>
<td>247 ± 25 (P &lt; 0.001)</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>133 ± 6</td>
<td>122 ± 5 (P &lt; 0.005)</td>
</tr>
</tbody>
</table>

did not affect renal blood flow, renal vascular resistance, glomerular filtration rate, heart rate, ventricular peak systolic pressure, rate of maximal change in intraventricular pressure during ventricular contraction, rate of maximal change in intraventricular pressure during ventricular relaxation, ventricular end diastolic pressure, ventricular diastolic minimal pressure, duration of ventricular contraction, duration of ventricular relaxation, time to one-half ventricular relaxation, time constant for ventricular relaxation, or heart rate × pressure product (data not shown).

At 26 weeks into the treatments, vasodilator responses in the mesentery were assessed. The decreases in mesenteric vascular resistance induced by acetylcholine were greater in 2-hydroxyestradiol-treated rats compared with control rats (Fig. 1). 2-Hydroxyestradiol did not significantly enhance vasodilation induced by sodium nitroprusside (data not shown) suggesting that enhancement of responses to acetylcholine was mediated by increased release of endothelial-dependent relaxing factors and/or reduced release of endothelial-dependent contracting factors.

**Discussion**

Our results indicate that 2-hydroxyestradiol markedly attenuates weight gain in an experimental model of obesity. Rats that received 2-hydroxyestradiol for approximately 6 months were on average 197 g lighter compared with control animals. This represents a 25% lower body weight in the 2-hydroxyestradiol-treated rats. In the present study, we selected to treat animals with 2-hydroxyestradiol from 12 weeks of age for approximately 6 months, rather than treat animals for 6 months after full development of obesity. This approach was taken because ZSF1 rats die prematurely of cardiovascular and renal disease, so a chronic study after the onset of obesity would have been confounded by significant mortality in the control group. This would have complicated the statistical analysis and threatened statistical validity. Therefore, the hypothesis that 2-hydroxyestradiol causes a reduction in body weight once obesity is well established was not tested in this study. Nonetheless, the results of the present study clearly indicate that 2-hydroxyestradiol attenuates the development of obesity in animals with a genetic predisposition for obesity.

The fact that 2-hydroxyestradiol attenuates the development of obesity in this particular model of obesity may be highly significant for human beings. The discovery of leptin (Zhang et al., 1994) raised hopes that administration of exogenous leptin would effectively control body weight in obese human beings. Unfortunately, subsequent studies in human beings revealed that the cause of human obesity is usually leptin resistance rather than leptin deficiency (Schwartz et al., 2000). In this regard, because the ZSF1 rat has leptin resistance due to a genetic defect in the gene coding for leptin receptors, agents that are effective in ZSF1 rats are likely to be effective in human beings.

2-Hydroxyestradiol prevented the development of obesity in ZSF1 rats at least in part by suppressing appetite. This is evident by the sustained decrease in food intake in 2-hydroxyestradiol-treated rats compared with control rats. The mechanism by which 2-hydroxyestradiol reduces appetite cannot be deduced from the current study. However, it is conceivable that 2-hydroxyestradiol acts in the hypothalamus to activate signal transduction mechanisms normally engaged by leptin receptors. It is also possible that 2-hydroxyestradiol may stimulate energy expenditure, and this possibility should be examined in future studies.

Leptin not only participates in the regulation of body...
weight, but also modulates insulin sensitivity (Harris, 2000). Accordingly, ZSF1 rats have insulin resistance and develop diabetes. Importantly, our results indicate that 2-hydroxyestradiol is an effective antidiabetic drug that attenuates the development of type II diabetes. In this regard, pretreatment with 2-hydroxyestradiol improves all measures, relative to age-matched untreated ZSF1 rats, of glucose control including glucosuria, polyuria, polydipsia, glucose tolerance, and glycated hemoglobin levels. The improved diabetic control induced by 2-hydroxyestradiol is further complemented by decreases in plasma cholesterol levels. Thus, 2-hydroxyestradiol attenuates the development of the metabolic syndrome.

Sequela of the metabolic syndrome include end stage renal disease and vascular disease. A major finding of the present study is that 2-hydroxyestradiol protects against proteinuria, a cardinal sign of diabetic nephropathy. 2-Hydroxyestradiol also enhances vascular responses to the endothelial-dependent vasodilator acetylcholine without significantly affecting responses to the endothelial-independent vasodilator sodium nitrouprusside. This suggests that 2-hydroxyestradiol alters signal transduction processes in vascular endothelium leading to increased release of endothelial-dependent relaxing factors and/or decreased release of endothelial-dependent contracting factors. This action of 2-hydroxyestradiol could account for, in part or in whole, the antihypertensive action of this compound.

Our extensive in vitro investigations of the biology of 2-hydroxyestradiol indicate that this metabolite of 17β-estradiol exerts powerful effects on vascular smooth muscle cells, glomerular mesangial cells, and cardiac fibroblasts (Dubey and Jackson, 2001a, 2001b). Indeed, the anti-growth effects of 17β-estradiol are mediated mostly by conversion of 17β-estradiol to catechol estrogens and methoxyestradiols, rather than via a direct effect of 17β-estradiol mediated via estrogen receptors (Dubey and Jackson, 2001a, 2001b). It is likely, therefore, that at least some of the beneficial effects of 2-hydroxyestradiol on renal and vascular function are mediated by a direct action of 2-hydroxyestradiol or its metabolite 2-methoxyestradiol on the kidneys and vasculature.

Empirical evidence indicates that the dose of 2-hydroxyestradiol used in the present study is nonestrogenic (Liu and Bachmann, 1998) as judged by the lack of effect of 2-hydroxyestradiol on uterine weight. There are at least two reasons for the low estrogenic activity of 2-hydroxyestradiol compared with estradiol. First, 2-hydroxyestradiol only weakly activates estrogen receptors (Gupta et al., 1998); second, the plasma clearance of 2-hydroxyestradiol is 10 times faster than that of 17β-estradiol (Ball et al., 1983). Thus, this agent may be safe to use in both males and females. Indeed, the present study employed male, rather than female, ZSF1 animals to determine whether 2-hydroxyestradiol would have efficacy in the male gender.

The results of this study have potential clinical relevance; however, in this regard, three caveats should be noted. First, because 2-hydroxyestradiol prevented the development of obesity and attenuated the development of the metabolic syndrome, it is not possible to discern whether the attenuation of nephropathy, vascular dysfunction, and hypertension by 2-hydroxyestradiol was a result of the prevention of obesity and the metabolic syndrome or was due in part to a direct action of 2-hydroxyestradiol on the kidneys and blood vessels. Most likely, both mechanisms contributed to the beneficial effects of 2-hydroxyestradiol; however, careful pair-fed studies are required to explore this issue. Second, it is unclear at this time whether tissue-specific partial estrogen agonist activity would limit the clinical usefulness of 17β-estradiol metabolites for obesity and the metabolic syndrome. Third, because the half-lives of 17β-estradiol metabolites are short, these drugs would have to be formulated in a sustained release form to be effective.

In conclusion, this study demonstrates that 2-hydroxyestradiol attenuates the development of obesity, attenuates the development of the metabolic syndrome, reduces nephropathy, and improves vascular endothelial function, all without adversely affecting left ventricular diastolic or systolic function. To our knowledge, no other anti-obesity drug has been discovered that exhibits a pharmacological profile as potentially beneficial as that observed for 2-hydroxyestradiol.

References


Bergman RN, Van Citters GW, Mittelman SD, Dea MR, Hamilton-Wessler M, Kim SP, and Eliner M (2001) Central role of the adipocyte in the metabolic syn-


Dubey RK, Gillespie DG, Jackson EK, and Keller PJ (1998) 17β-estradiol, its metab-


Kernan WN, Viscoli CM, Brass LM, Broderick JP, Brett T, Feldman E, Morgen-


Oparil S, Levine RL, Chen SJ, Durand J, and Chen YP (1997) Sexually dimorphic response of the balloon-injured rat carotid artery to hormone treatment. *Circula-
tion* 95:1301–1307.


Address correspondence to: Dr. Edwin K. Jackson, Center for Clinical Pharmacology, University of Pittsburgh, 623 Scaife Hall, 3550 Terrace Street, Pittsburgh, PA 15261. E-mail: edj@pitt.edu