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

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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 effect 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 phar-
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, urine 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, water intake, urine volume, urinary glucose excretion, urinary protein excretion, fasting plasma glucose levels, plasma glucose levels 2 h after an oral glucose load of 2 g/kg, glycated hemoglobin, and plasma cholesterol.

Treatment with 2-hydroxyestradiol for 26 weeks significantly reduced mean arterial blood pressure (Table 2), but

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rats Randomized to Vehicle</th>
<th>Rats 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 1 Values of parameters at baseline (12 weeks old and before initiation of treatments) in ZSF1 rats. Values represent means ± S.E.M. for nine to ten rats in each group.
TABLE 2
Values of parameters after 24 to 26 weeks of treatment with either vehicle (control) or 2-hydroxyestradiol in ZDF1 rats
Values represent means ± S.E.M. for nine to ten rats in each group. *P* values were calculated with an unpaired Student’s *t* test.

<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 (<em>P</em> &lt; 0.001)</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>44.6 ± 1.0</td>
<td>36.6 ± 0.9 (<em>P</em> &lt; 0.001)</td>
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<tr>
<td>Water intake (ml/day)</td>
<td>131.8 ± 7.0</td>
<td>67.4 ± 4.5 (<em>P</em> &lt; 0.001)</td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
<td>86.5 ± 3.0</td>
<td>45.6 ± 3.8 (<em>P</em> &lt; 0.001)</td>
</tr>
<tr>
<td>Urinary glucose excretion (mg/day)</td>
<td>6.5 ± 0.4</td>
<td>1.7 ± 0.5 (<em>P</em> &lt; 0.001)</td>
</tr>
<tr>
<td>Urinary protein excretion (mg/day)</td>
<td>586 ± 41</td>
<td>333 ± 21 (<em>P</em> &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 (<em>P</em> &lt; 0.001)</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 (<em>P</em> &lt; 0.001)</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>12.5 ± 1.7</td>
<td>3.8 ± 0.7 (<em>P</em> &lt; 0.001)</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/100 ml)</td>
<td>399 ± 24</td>
<td>247 ± 28 (<em>P</em> &lt; 0.001)</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>133 ± 6</td>
<td>122 ± 5 (<em>P</em> &lt; 0.001)</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 19.7 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 rather than leptin deficiency, 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 endothelium-dependent vasodilator acetylcholine without significantly affecting responses to the endothelium-independent vasodilator sodium nitroprusside. This suggests that 2-hydroxyestradiol alters signal transduction processes in vascular endothelium leading to increased release of endothelium-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 catecholestriadiols and methoxyoestradiols, 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-methoxyoestradiol 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 in part due 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**


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