Protective effects of estrogen on gender-specific development of diet-induced hypertension

CHRISTIAN K. ROBERTS,1 NOSRATOLA D. VAZIRI,2 AND R. JAMES BARNARD

1Department of Physiological Science, University of California, Los Angeles 90095; and 2Division of Nephrology and Hypertension, Departments of Medicine, Physiology and Biophysics, University of California, Irvine, California 92697

Received 30 May 2001; accepted in final form 6 July 2001

Roberts, Christian K., Nosratola D. Vaziri, and R. James Barnard. Protective effects of estrogen on gender-specific development of diet-induced hypertension. J Appl Physiol 91: 2005–2009, 2001.—Dietary and humoral factors are thought to be involved in the development of hypertension. This study investigated the interaction between diet and gonadal hormone status in the development and reversibility of hypertension. Normal male and female and ovariectomized (OVX) female Fischer rats were placed on either a high-fat (primarily saturated), refined carbohydrate (sucrose) (HFS) or a low-fat, complex carbohydrate (LFCC) diet at 2 mo of age, and body weight and systolic blood pressure (BP) were measured. Male and OVX female rats were initially on the diets for 7 mo, whereas normal female rats were on the diets for 2 yr. After this initial phase, a group of rats from each of the normal HFS groups were converted to the LFCC diet for a period of 1 mo (males) and 2 mo (females). The OVX females were subcutaneously implanted with a 0.5-mg estradiol (E2) pellet for 1 mo. A significant rise in arterial BP occurred within 12 mo in female and only 2 mo in male rats on the HFS diet, exceeding 140 mmHg after 24 and 7 mo, respectively. Conversion from the HFS to the LFCC diet led to a normalization of BP in both female and male rats. HFS diet-induced hypertension was accelerated by OVX in female rats, approaching the pattern seen in male rats. The effect of OVX was completely reversed by E2 replacement. BP did not significantly change in any of the LFCC groups at any time point, and E2 replacement had no effect on BP in the OVX LFCC group. All HFS groups had significantly greater body weight, with differences occurring sooner in the male and OVX rats compared with the female rats. Diet modification resulted in a partial but significant reduction of body weight, but E2 replacement did not. These results demonstrate that long-term consumption of HFS diet induces hypertension in both genders and is reversible by diet modification. Hypertension is significantly delayed in females with functional ovaries. This protection is lost by OVX and restored by estrogen replacement. Thus hormone status contributes to the delayed onset of diet-induced hypertension in females compared with males.

HYPERTENSION IS THE MOST COMMON cardiovascular disease in the United States and is a hallmark risk factor for coronary artery disease, stroke, and congestive heart failure (15). It has been estimated that one-quarter of all adults and one-half of all individuals over the age of 65 have hypertension (systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg) in the United States and only 47% have optimal blood pressure (<120/80 mmHg) (1, 5). The incidence of hypertension is increasing, and it is more common in men than in women (1, 5, 15). Specifically, men start to show a significant rise in the incidence of hypertension in the teenage years, whereas women generally do not experience significant hypertension until after menopause (15, 31). Furthermore, it has been estimated that one-third of U.S. women 20–74 yr of age have hypertension or are administered antihypertensive medications (16a). The mechanisms for the gender-specific differences in the development of hypertension are not completely understood; however, hormonal differences (i.e., estrogen, androgens) appear to play a role.

Components of a typical Western diet, characterized by high saturated fat and refined carbohydrate, also appear to be involved in the development of hypertension. Several recent studies (9, 25, 34), including several from our own laboratory (23, 24), have suggested that dietary factors can induce endothelial dysfunction and hypertension. Specifically, it has been recently documented that long-term consumption of a Western diet induces oxidative stress and hypertension in normal female rats (24). On the basis of these data and the notion that there may be hormone-related differences in the time course of blood pressure elevation, and 3) diet-induced hypertension can be ameliorated by either diet or estradiol (E2) replacement.

METHODS

Animals and diet. All protocols were conducted in accordance with the University of California, Los Angeles, Animal...
Research Committee. Inbred male, female, and ovariectomy-
mized (OVX) female Fischer rats were obtained from Harlan
Sprague Dawley (San Diego, CA) at 2 mo of age. This rat
model has been used in previous studies in our laboratory, as
the female Fischer rat normally shows little weight gain after
its maturation phase (3, 4). The rats were randomly assigned
to either the low-fat, complex carbohydrate (LFCC) or high-
fat (primarily saturated), refined carbohydrate (sucrose) (HFS) diet, housed four per cage with a 12-h light cycle
starting at 0700 at 75°–76°F, and fed ad libitum with bowls
placed in the cages to ensure that all animals had access to
the food. The diets were prepared in powder form by Purina
test diets (Richmond, IN) and contained a standard vitamin
and mineral mix and all essential nutrients. The LFCC diet
(Purina 5001) is low in saturated fats and contains mostly
complex carbohydrates (starch), whereas the HFS diet is
high in saturated and monounsaturated fats (primarily from
lard plus a small amount of corn oil) and high in refined
sugar (sucrose) as previously published (23). After 2 yr (fe-
male rats) and 7 mo (male rats) on the HFS diet, some of
the animals were switched to the LFCC diet for a period of 2 mo
(females) or 1 mo (males). In addition, the rats were weighed
weekly.

E2 replacement. Once hypertension had been established
in the OVX HFS rats at 7 mo, some of the OVX rats were
given E2 replacement therapy for 1 mo by subcutaneous
pellet (0.5-mg pellets, 60-day pellets, Innovative Research,
Sarasota, FL). This dose is designed to achieve a serum level
of E2 in the range of 50–100 pg/ml.

RESULTS

Body weight. Body weight data are shown in Table 1. For
the female rats, body weight was significantly
greater in the HFS group after week 20. For the male rats,
body weight became significantly greater in the
HFS group after week 3. For the OVX rats, body weight
was greater in the OVX LFCC group compared with
the normal LFCC group at randomization and greater
in the OVX HFS group compared with the HFS group
after week 1. In addition, body weight was significantly
greater by week 6 in the OVX HFS group compared with
the OVX LFCC group. After the 2-mo and 1-mo
conversion to the LFCC diet, respectively, body weight
was significantly reduced in both female and male rats
but did not reach the value found in the LFCC rats
(P < 0.05, ANOVA; Table 1). Body weight did not
decrease significantly after 1 mo of E2 replacement.

Blood pressure. Systolic blood pressure data for the
female groups are depicted in Fig. 1. Systolic blood
pressure was significantly elevated in the HFS com-
pared with the LFCC rats after 1 yr (P < 0.01). Systolic
blood pressure exceeded 140 mmHg in all HFS female
animals by 2 yr but remained normal in the LFCC

Table 1. Effect of gender and diet on body weight

<table>
<thead>
<tr>
<th></th>
<th>Randomization</th>
<th>2 mo</th>
<th>6 mo</th>
<th>12 mo</th>
<th>24 mo</th>
<th>26 mo</th>
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<tbody>
<tr>
<td>Normal female rats</td>
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<tr>
<td>LFCC</td>
<td>124.9 ± 2.0</td>
<td>156.6 ± 3.5</td>
<td>188.0 ± 1.4</td>
<td>218.5 ± 2.5</td>
<td>260.0 ± 6.0</td>
<td>259.0 ± 4.9</td>
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<tr>
<td>HFS</td>
<td>125.3 ± 2.0</td>
<td>158.3 ± 3.2</td>
<td>214.0 ± 3.4†</td>
<td>300.2 ± 7.0*</td>
<td>374.0 ± 9.0*</td>
<td>336.0 ± 10.7*</td>
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<tr>
<td>HFS → LFCC</td>
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<tr>
<td>Normal male rats</td>
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<tr>
<td>LFCC</td>
<td>222.7 ± 4.7</td>
<td>245.4 ± 5.5</td>
<td>294.2 ± 8.9</td>
<td>345.6 ± 10.5</td>
<td>376.9 ± 9.2</td>
<td>418.9 ± 10.5</td>
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<tr>
<td>HFS</td>
<td>228.4 ± 4.1</td>
<td>265.9 ± 7.7*</td>
<td>329.8 ± 7.4*</td>
<td>410.5 ± 11.5*</td>
<td>448.3 ± 11.4</td>
<td>519.5 ± 18.5*</td>
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<tr>
<td>HFS → LFCC</td>
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<td>Ovariectomized female rats</td>
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<tr>
<td>OVX LFCC</td>
<td>146.7 ± 1.7</td>
<td>181.3 ± 2.9</td>
<td>198.2 ± 2.7</td>
<td>227.6 ± 3.2</td>
<td>254.1 ± 2.9</td>
<td>269.2 ± 4.3</td>
</tr>
<tr>
<td>OVX HFS</td>
<td>146.4 ± 3.4</td>
<td>190.6 ± 5.3</td>
<td>229.8 ± 4.1*</td>
<td>266.8 ± 4.7*</td>
<td>285.6 ± 3.9*</td>
<td>299.1 ± 4.5*</td>
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<td>OVX LFCC + E2</td>
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<td>OVX HFS + E2</td>
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Values are means ± SE for n = 8 in all groups. HFS → LFCC, conversion from high-fat, refined carbohydrate (sucrose) (HFS) diet to
low-fat, complex carbohydrate (LFCC) diet; OVX, ovariectomized; E2, estradiol. *P < 0.001 vs. corresponding LFCC group; †P < 0.05 vs.
LFCC group; ‡P < 0.01 vs. LFCC group.
animals. Within 2 mo after conversion to the LFCC diet, blood pressure in the formerly hypertensive female HFS group returned to normal.

Systolic blood pressure data for the male groups are depicted in Fig. 2. Blood pressure significantly increased after 2 mo in the male rats consuming the HFS diet compared with 12 mo in their female counterparts. As with the female rats, blood pressure remained unchanged throughout the observation period in the male rats consuming the LFCC diet. No significant difference in blood pressure was found between the male and female rats consuming the LFCC diet. After 1 mo of conversion to the LFCC diet, blood pressure returned to normal in the formerly hypertensive HFS male rats.

The blood pressure measurements in the OVX groups are depicted in Fig. 3. Blood pressure was significantly greater in the OVX HFS group compared with that in the OVX LFCC group after 7 mo. Blood pressure exceeded 140 mmHg in all OVX HFS animals at 7 mo. Although blood pressure was higher in the OVX LFCC group compared with the LFCC group, this difference was not statistically significant. E\textsubscript{2} replacement normalized blood pressure in the OVX HFS group but had no effect in the OVX LFCC group.

**DISCUSSION**

We recently documented that prolonged consumption of a diet high in fat and refined sugar by normal female rats induces marked oxidative stress, a decrease in bioavailable nitric oxide (NO), and hypertension, independent of excess energy intake (24). Others have also noted that dietary factors can affect blood pressure. Dobrian et al. (9) documented that male rats fed a Western diet for 10 wk developed hypertension. Yoshioka et al. (34) documented that chronic lard feeding increased systolic blood pressure in the absence of excessive energy intake. In addition, Shinozaki et al. (25) fed animals a high-fructose diet and reported endothelial dysfunction and elevated reactive oxygen species generation. Finally, Vogel et al. (32) reported that a single high-fat meal can induce transient endothelial dysfunction. We undertook the present study to determine whether diet could also induce hypertension in male rats and, if so, whether there is a difference in the time course and/or magnitude of the rise in blood pressure. We noted the development of diet-induced hypertension in both female and male rats. Conversion from the HFS to the LFCC diet for a period of 1 mo in males and 2 mo in females resulted in a reversal of hypertension. The Dietary Approaches to Stop Hypertension clinical trial (2) documented that a diet low in refined sugar with reduced saturated fat and high fruit and vegetable intake decreased blood pressure in both hypertensive and normotensive individuals.

The fact that the incidence of cardiovascular diseases increases after menopause suggests that estrogen plays a protective role in the cardiovascular system. Only one in nine women has clinical evidence of coronary disease in the 45- to 65-year of age range; however, this increases to one in three in those older than 65 years of age (16a). Epidemiological studies have documented that estrogen replacement is associated with a reduction in the incidence of coronary events in postmenopausal women (29). Cross-sectional data have also documented higher blood pressures after menopause (28). However, the influence of menopause on blood pressure is not without controversy. For instance, the Framingham data (13) showed no rise in blood pressure with menopause. Use of oral contraceptives pills has in some cases been associated with an increased blood pressure (28). Animal studies have also produced discordant results, possibly stemming from the differences in experimental design, species, and doses utilized (7, 16).

The present study determined the role of gonadal hormone status in the development of diet-induced hypertension. Despite the lack of estrogen in the OVX LFCC group for 7 mo, no increase in blood pressure was noted. This suggests that, in the absence of estrogen, a LFCC diet allows for maintenance of normal blood pressure. The normal blood pressure also noted in the male LFCC group supports this contention. However, estrogen deprivation in the OVX HFS animals markedly accelerated the onset of diet-induced hypertension in female rats and simulated the rise in blood pressure noted in male rats. In addition, the observation that the blood pressure differences were greatest in the HFS-fed female rats after menopause

![Fig. 2. Systolic blood pressure of normal male rats placed on the LFCC and HFS diets at 2 mo of age and of the animals converted to the LFCC diet for 1 mo (open bar); n = 8 observations per group. *P < 0.01 vs. LFCC.](image)

![Fig. 3. Systolic blood pressure of ovariectomized (OVX) female rats placed on the LFCC and HFS diets at 2 mo of age and of the animals replaced with estradiol (E\textsubscript{2}) for 1 mo (OVX+E\textsubscript{2}); n = 8 observations per group. *P < 0.01 vs. OVX-LFCC.](image)
suggests a possible protective role for estrogen and loss of this protection with menopause. As noted, a similar pattern is observed in women in the United States, where hypertension is generally not seen until after menopause, and suggests that this dietary paradigm may be applicable to human hypertension. De Meersman et al. (8) demonstrated a decrease in both resting and isometric-exercise systolic and diastolic blood pressures in postmenopausal women receiving estrogen replacement therapy (ERT), which they attributed to enhanced arteriolar distensibility, since ERT had no effect on cardiac output. Evidence suggests that estrogen has a direct effect on the vascular wall, as it has been noted that it increases transcription of the endothelial NO synthase gene (14) and increases NO synthase activity (33). Data from acute infusion of E2 to postmenopausal women (11) and chronic ERT in both OVX rats (27) and postmenopausal women (10) have shown improved endothelium-dependent relaxation. Hayashi et al. (12) found that female rabbit aortic rings do indeed produce greater basal amounts of NO compared with males and OVX females. Furthermore, estrogen deprivation in normotensive women induces oxidative stress and endothelial dysfunction (30). Interestingly, we have shown that a HFS diet can also induce oxidative stress (24) and endothelial dysfunction (22), leading to hypertension. Thus diet may compound the effects of hormones on blood pressure. The fact that both diet (this study) and estrogen deprivation alone induce hypertension and together induce more rapid hypertension supports this contention.

The role of androgens in the regulation of blood pressure is also unclear. Young OVX spontaneously hypertensive rats (SHR) do not show an increase in blood pressure; however, testosterone administration increases blood pressure in these animals. Furthermore, castration decreases blood pressure in male SHR to the level of female SHR (20). Because the increase in blood pressure in postmenopausal women takes 5–20 yr to develop (5), lack of female hormone may not be the only contributing factor for the elevated blood pressure. The shorter time course of hypertension development in the males compared with the normal females in the present study may be, in part, related to elevated androgens in the former group. Furthermore, consistent with the increase in oxidative stress and decrease in bioavailable NO noted in the present model (24), Reckelhoff (19) has proposed a scheme by which androgens increase blood pressure by stimulating secretion. This increases angiotensin II levels, which promotes vasoconstriction directly and indirectly by increasing the generation of superoxide, leading to enhanced NO inactivation. Along these lines, Nickenberg et al. (18) demonstrated that estrogen decreases angiotensin receptor 1 gene expression. Furthermore, treatment of intact SHR with an androgen receptor antagonist decreases blood pressure (21). Confounding the issue is the recent finding from Nathan et al. (17) that castrated male mice fed a Western diet have an increase in atherogenic lesion formation, but testosterone supplementation reduces lesion formation, whereas aromatase inhibition reverses the protective effect, suggesting an anti-atherogenic role of testosterone due to conversion to estrogen in endothelial cells. Evidence that both diet (C. K. Roberts, R. J. Barnard, Z. Ni, and N. D. Vaziri, unpublished observations) and androgens (26) lower brain neuronal NO synthase expression and/or activity whereas estrogen increases brain NO synthase activity (33) supports the contention that diet and androgens may have an additive effect on the development of hypertension, as it is well known that NO decreases sympathetic outflow.

Overall, diet-induced hypertension is affected by hormone status. Males and OVX females display marked hypertension much earlier in their lifespan than intact females, and estrogen and androgens appear to play a role in the time course differences noted. Conversion to a LFCC diet normalized blood pressure, independent of hormone status. In addition, hormone replacement in females was also able to normalize blood pressure. Because body weight did not significantly change in the E2-replaced OVX HFS group, estrogen apparently does not normalize diet-induced changes in blood pressure through changes in body weight. In addition, obesity does not appear to be a major contributing factor in this model, as the OVX LFCC group weighed significantly more than normal LFCC animals, without exhibiting elevated blood pressure. Thus a LFCC diet appears to mitigate the changes in blood pressure that occur with aging and different hormonal profiles and may be effective in the prevention and treatment of hypertension.

This study was supported by National Institute on Aging Grant AG-05792 and a grant from the L-B Research/Education Foundation. C. K. Roberts is supported by a postdoctoral fellowship from the American Heart Association, Western States Affiliate.

REFERENCES