ABSTRACT  Sex hormones and the selective estrogen receptor modulator tamoxifen affect food consumption and body weight in normotensive rats. This study investigated the effects of hormone manipulation and tamoxifen on weight gain and food intake in the presence of chronic systemic hypertension. Male and female spontaneously hypertensive rats (SHR) were either neutered or sham operated before puberty, and subgroups of neutered females received either estrogen replacement therapy (ERT) or tamoxifen at the age of 12 wk. Weekly body weight and food consumption were assessed, and food consumption was normalized to metabolic weight (g body$^{-2/3}$). Neutering reduced weight gain in males ($P = 0.0001$), but increased it in females ($P < 0.0001$). Both ERT and tamoxifen treatment prevented this increase in weight, with body weight dropping to levels of sham-operated rats for ERT, whereas rats given tamoxifen maintained greater body weights than sham-operated rats ($P < 0.0001$). This contrasts with previous work in normotensive females in which sham-operated and tamoxifen-treated females did not differ. Neutering normalized food consumption relative to sham-operated rats in both males and females ($P < 0.05$). Although ERT returned it to normalized intakes of sham-operated rats, tamoxifen reduced normalized food consumption relative to that of both sham-operated and ERT groups. In hypertensive rats, body weight is modulated by sex hormones in both males and females, but in opposite directions. Both estrogen and tamoxifen exert immediate effects in females. Interestingly, the effect of tamoxifen on body weight appears to be greater in hypertensive than in normotensive rats. J. Nutr. 132: 2246–2250, 2002.

KEY WORDS: • spontaneously hypertensive rats • gender • 17β-estradiol • selective estrogen receptor modulator

Steroid hormones affect body weight and food consumption, and the hypophagic effects of estradiol, attributed to the direct effects on the hypothalamus, are well documented (1,2). Recent studies in normotensive rats showed that these steroid effects are important throughout growth from puberty to adulthood, and that gender differences exist (3). To date, the majority of studies into the effects of steroid hormones on body weight have been conducted in normal rats. However, there is a well-established and strong association between increased body weight and hypertension (1). In North America, the prevalence of systemic hypertension in the adult population is >20% (4), representing millions of individuals. To date, there has not been an investigation into whether existing hypertension alters growth from puberty, and whether this is affected by sex hormones. What effects steroids exert on body weight profiles and food consumption in the presence of hypertension are unknown.

Selective estrogen receptor modulators (SERM)$^3$ such as tamoxifen are potent nonsteroidal antiestrogenic agents that are utilized in the treatment of estrogen-responsive breast cancer and may be useful as preventative agents in the development of this disease. Previous studies showed that tamoxifen exerts estrogenic effects on the growth of normotensive rats (3). As with the effects of steroids on growth and food consumption in the presence of hypertension, the effect of SERM on body weight and food consumption in the presence of pathologic conditions such as chronic systemic hypertension is unknown. Therefore, this study was conducted to document the weekly profile of the effect of male and female sex hormones and the SERM tamoxifen on weight gain and food intake of hypertensive rats throughout the developmental stages, and to determine whether these effects are transitory or sustained.

MATERIALS AND METHODS

Age-matched male and female spontaneously hypertensive rats (SHR) were used for these studies (Charles River Laboratories, St. Constant, Canada). Both genders have dramatically higher systemic blood pressures (>200/150 mm Hg) than control rats (5), confirming the presence of hypertension. Rats were treated in accordance with Canada Council and NIH guidelines. All rats were housed separately and were allowed free access to water. The rats consumed ad libitum a commercial nonpurified diet [Purina Rat Chow 5001, Ralston

1 Funded by a grant from the Heart and Stroke Foundation of Ontario (# T4723). W.J.W. was supported by the Medical Research Council of Canada/K. M. Hunter Doctoral Research Award.

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3 Abbreviations used: ERT, estrogen replacement therapy; Neut, surgically neutered males and females; SERM, selective estrogen receptor modulator; Sham, sham-operated; SHR, spontaneously hypertensive rats; Tam, neutered + tamoxifen.
RESULTS

In neutered female rats, plasma concentration of 17β-estradiol was virtually undetectable (<4 pmol/L), whereas intact females (73 ± 37 pmol/L) and neutered females receiving ERT (92 ± 19 pmol/L) had similar concentrations. Neutered female rats receiving tamoxifen had undetectable (<4 pmol/L) plasma 17β-estradiol. Plasma testosterone levels were 4 ± 2 nmol/L in intact males.

For the ages up to 12 wk, neutered males weighed ~10% less than sham-operated males (P < 0.0001) throughout this period, and both groups gained weight with time (Fig. 1A). The slopes of the growth curves did not differ between sham-operated (16.6 ± 1.9 g/wk) and neutered (15.2 ± 1.1 g/wk) males. Growth curves of sham-operated and neutered females to 12 wk, before the initiation of hormone replacement, are shown in Figure 1B. Throughout this period, neutered females weighed more than sham-operated females (P < 0.0001). Both sham-operated and neutered females gained body weight up to 12 wk of age, with the slope of the growth curve 36% steeper in neutered females (11.3 ± 0.4 g/wk) than in the sham-operated females (8.3 ± 0.4 g/wk, P < 0.001). By 12 wk of age, neutered females were 19% heavier than intact females (P < 0.0001).

In females, weekly food consumption, (in some cases exceeding P < 0.0001) did not differ between sham-operated and neutered groups (P = 0.15), with both groups decreasing food intake ~20% between 9 and 12 wk (Fig. 2A). When food consumption was normalized to metabolic weight (Fig. 2B), neutered females tended to have lower intakes than sham-operated females, although this was significant only at wk 9 (P < 0.05).

Growth curves for males and females between wk 12 and 20 are shown in Figure 3A and B, respectively. The wk 12 values are included to enable comparison of pretreatment data with post-treatment data. Both neutered and sham-operated males grew continuously and rapidly throughout this period. However, neutered males weighed less than sham-operated males by as much as 10% (P < 0.0001). Between wk 12 and 20, the slope of the growth curve for neutered males (8.6 ± 0.2 g/wk) was 18% shallower than that of the sham-operated males (10.5 ± 0.6 g/wk, P < 0.01). These slopes were ~40% lower than those for the 8- to 12-wk period in for both sham-operated and neutered groups.

Both sham-operated and neutered females continuously
gained weight between 12 and 20 wk by 14–16% (Fig. 3B). Neutered females weighed more than sham-operated females throughout this period (P < 0.0001). Neutered females receiving ERT did not increase in weight after the initiation of treatment, and tended to weigh less by wk 20 (P = 0.24). Neutered females receiving ERT and sham-operated females did not differ in body weight; however, compared with neutered females, those receiving ERT were as much as 24% smaller after the initiation of treatment (P < 0.0001). Neutered females receiving tamoxifen did not gain weight after the initiation of treatment and had body weights that were intermediate between those of neutered females (P < 0.0001), and both sham-operated females and neutered females receiving ERT (P < 0.0001).

For wk 12–20, the slope of the growth curve of neutered females (4.8 ± 0.4 g/wk) was 45% steeper than that of sham-operated females (3.3 ± 0.3 g/wk, P < 0.05). The slope of the growth curve of ERT females (−2.5 ± 0.3 g/wk) was less than that of either neutered or sham-operated females (P < 0.0001) throughout this period. Neutered females receiving tamoxifen had a growth curve (0.8 ± 0.1 g/wk) that was flatter than those of both sham-operated and neutered females (P < 0.0001), but steeper than that of neutered females receiving ERT (P < 0.0001). For all groups of females, these slopes were essentially flat, indicating a cessation of rapid growth for wk 12–20.

Weekly food consumption in neutered males was 11–17% lower than that of sham-operated males between wk 12 and 20 (P < 0.0001) (Fig. 4A). Only neutered males showed modest fluctuations in weekly food consumption at wk 14 and 16. Sham-operated and neutered females generally consumed more food than the groups of neutered females receiving ERT or tamoxifen (P < 0.05). Neutered females receiving ERT generally consumed less food than both sham-operated and neutered females throughout this period (Fig. 4B). As seen in the males, there tended to be modest fluctuations (P = 0.17) in weekly food consumption across time in the females. Interestingly, the neutered females receiving ERT showed a 14% reduction immediately after initiation of hormone replacement, which returned to wk 12 values by wk 18, followed by

FIGURE 3  Body weights of sham-operated or neutered male (A) and female (B) spontaneously hypertensive rats (SHR) age 12–20 wk. Rats were either sham-operated or neutered at 17–21 d. Subgroups of neutered females received either 17β-estradiol replacement (ERT) or tamoxifen (TAM) beginning at 12 wk (arrow). Values are means ± SEM, n = 6 sham; 9 neutered (males) and n = 6 sham; 20 neutered (females). aDifferent from sham-operated at the same age, P < 0.05. bDifferent from the previous week, P < 0.01.

FIGURE 2  Absolute (A) and normalized to metabolic body weights (g body2/3) (B) food intakes of sham-operated or neutered male spontaneously hypertensive rats (SHR) age 9–12 wk. Rats were operated on at 17–21 d. Values are means ± SEM, n = 6 sham; 9 neutered (males) and n = 6 sham; 20 neutered (females). aDifferent from sham-operated at the same age, P < 0.05. bDifferent from the previous week, P < 0.01.
a second 13% drop by the end of the study. No such drops in food consumption occurred in sham-operated or neutered females. Neutered females receiving tamoxifen also showed an immediate 10% reduction in food consumption, which continued until wk 18, after which food consumption increased. Tamoxifen-treated females tended to have the lowest weekly food consumption of all four groups of females ($P < 0.05$).

**DISCUSSION**

The current study investigated in rats the effects of alterations in sex hormones and the SERM tamoxifen in the presence of chronic systemic hypertension on body weight and food consumption during growth and maturation. Neutering both male and female rats before puberty had significant but opposite effects on growth. In males, neutering reduced body weight, which was associated with reduced food consumption. In contrast, neutering increased body weight in female rats, but this was associated with a reduction in food consumption.

When weekly food consumption was normalized to metabolic weight (g body$^{-2/3}$), neutered males consumed up to 14% less than sham-operated males throughout this period ($P < 0.01$) (Fig. 5A). Both groups of males showed a gradual 11–15% decline in normalized food consumption. Despite similar normalized food consumptions in neutered females and sham-operated females initially, neutered females consumed less than sham-operated females after wk 12 (Fig. 5B). Sham-operated females had the highest normalized food consumption throughout the study. After an immediate 14% drop in normalized food consumption ($P < 0.01$), neutered females receiving ERT had intakes similar to sham-operated females until wk 20. Neutered females receiving tamoxifen had ~20% lower normalized food consumption than sham-operated females ($P < 0.01$), and had generally lower normalized intakes than females receiving ERT. All four groups of females showed reductions in normalized food consumption between wk 12 and 20.

**FIGURE 5** Food consumption normalized to metabolic weight of male (A) and female (B) spontaneously hypertensive rats (SHR) age 12–20 wk. Subgroups of neutered females received either 17β-estradiol replacement (ERT) or tamoxifen (TAM) beginning at 12 wk (arrow). Rats were operated on at 17–21 d. Values are means ± SEM, n = 6 sham; 9 neutered (males) and n = 6 sham; 6 neutered (females), 7 ERT, 7 TAM. aDifferent from sham-operated at the same age, $P < 0.05$ (in some cases exceeding $P < 0.0001$). bDifferent from neutered at the same age, $P < 0.05$. cDifferent from ERT at the same age, $P < 0.05$. dDifferent from the previous wk, $P < 0.05$ (in some cases exceeding $P < 0.0001$).
normalized to body weight compared with intact females. Replacement of estrogen in neutered females was associated with an immediate and sustained reduction in body weight, returning it to sham-operated levels. A cessation in growth was also seen when neutered females received Tam, but no reduction as occurred with ERT. In addition, food consumption was affected dramatically by ovarian hormones and the SERM tamoxifen, with estrogen returning normalized intake to sham-operated levels, but tamoxifen generally reducing it.

Slopes of growth curves of sham-operated and neutered males for wk 9–12 and 12–20 are comparable to those previously reported in normotensive males of a similar age (3), suggesting that chronic hypertension does not affect the rate of growth to adulthood in male rats. The 53% reduction in slope after wk 12 in the hypertensive SHR is also comparable to that observed in the normotensive males. Interestingly, when normalized to metabolic weight, neutered hypertensive males consumed less food than sham-operated males, in agreement with previous studies using normotensive males.

There are numerous studies in both animals and humans showing that alterations in body composition occur when ovarian hormones are removed; this may contribute to the alterations in weight. These changes include a redistribution in body fat to the skin with neutering (7), and a higher fat content and lower lean tissue mass (8). As was observed in normotensive rats, neutered hypertensive females also showed a significant and sustained increase in body weight, which may be associated with these alterations in body composition. In the SHR females after 9 wk and before 12 wk of age, there was no difference in normalized food consumption between sham-operated and neutered females despite the differences in body weight. This effect was also seen in normotensive WKY females. Both normotensive and hypertensive females had similar food intakes up to this age.

Upon initiation of estradiol replacement to neutered SHR females, an immediate cessation and an actual reduction of growth occurred, so that there was no difference between ERT females and sham-operated females. This is comparable to the results in normotensive females. This cessation in growth was accompanied by an immediate but transient stop in food consumption, which then returned to pretreatment values. Ultimately, sham-operated and ERT females generally had similar body weights and normalized food intakes. Thus, the effects of the absence of ovarian hormones on the increased growth was corrected by the administration of 17β-estradiol, which prevented further weight gain and maintained relative food intakes at levels of intact females. In contrast, despite continuous increases in body weight, normalized food consumption in neutered females dropped steadily until the end of the study. This suggests that either neutered females were expending less energy or were more efficient at utilizing food energy, and that this was not affected by the presence of hypertension because similar effects were seen in normotensive females (3).

Initiation of tamoxifen treatment to neutered females also was associated with body weight reaching an immediate plateau after the initiation of treatment at levels that were significantly greater than those of intact sham-operated females. This is in contrast to normotensive female rats in which there was no difference in body weight between sham-operated and tamoxifen-treated groups. Thus, because body weight profiles were not the same in sham-operated and tamoxifen-treated hypertensive females, the estrogen-like effects of tamoxifen on weekly weight changes appeared to be blunted by chronic systemic hypertension. The reasons for this are unclear. Tamoxifen appears to be less potent than estradiol because neutered SHR females receiving estradiol had significantly lower body weights than those receiving tamoxifen. Despite the greater body weights in tamoxifen-treated females, their normalized food consumption was lower than that of those receiving estradiol. Again, this suggests that hypertension modulates the influence of tamoxifen because ERT and sham-operated females had similar normalized food intakes. Previous studies showed that tamoxifen administration to both intact and ovariectomized female mice was associated with dramatic elevations in the activities of uterine 17β-hydroxysteroid dehydrogenase (9), responsible for the conversion of estrone to estradiol. This suggests that localized changes in estrogen levels may account in part for the estrogenic effects of tamoxifen. It is possible that systemic hypertension reduces the ability of tamoxifen to regulate the activity of enzymes important in steroid metabolism, possibly accounting for the reduced estrogen-like effects observed in female SHR.

In conclusion, this study demonstrated that in male and female rats with chronic systemic hypertension, weight gain and food consumption are modulated by sex hormones. As previously reported in normotensive females, estrogen exerted a dramatic effect on body weight and food consumption. However, the effects of the SERM tamoxifen were modulated by the presence of hypertension because body weights were significantly greater than those of intact females, which was not seen in normotensive females. Furthermore, the estrogen-like effects of tamoxifen on the uterus appeared to be enhanced by hypertension. These data suggest that the use of tamoxifen in hypertensive females may alter food intake and body weight profiles differently than in normotensive females.

ACKNOWLEDGMENTS

The authors thank Warren Ball and Evelyn Bulczynski for looking after the rat colonies detailed in this study.

LITERATURE CITED