

Differential Susceptibility to Obesity Between Male and Female Mice

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ABSTRACT

Objective: The prevalence of obesity has increased dramatically in the United States. A direct comparison in the predisposition to obesity between men, premenopausal women, and postmenopausal women has not been made. To determine the effects of gender and ovarian hormones on the susceptibility to obesity, we conducted laboratory studies with mice. To eliminate confounders that can alter body weight gain, such as age and food consumption; we used animals that were the same age and controlled the amount of calories they consumed.

Methods: We determined gender-specific susceptibility to obesity between male, non-ovariectomized female, and ovariectomized female mice. To compare susceptibility to gaining body weight between males and females, animals from each sex were exposed to either a 30% calorie-restricted, low-fat (5% fat), or high-fat (35% fat) diet regimen. To establish the role of ovarian hormones in weight gain, the ovaries were surgically removed from additional female mice, and then those mice were exposed to the diets described above. Percent body fat and percent lean mass in the mice were determined by dual energy x-ray absorptiometry (DEXA).

Results: In all three diet categories, male mice had a greater propensity of gaining body weight than female mice. However, ovariectomy eliminated the protection of female mice to gaining weight; in fact, ovariectomized female mice patterned like male mice in their susceptibility to weight gain. Results show that male mice are more likely to become obese than female mice and that the protection against obesity in female mice is eliminated by ovariectomy.

Conclusion: Understanding metabolic differences between men and women will allow us to discover better preventive and treatment strategies for diseases associated with body weight such as cancer and cardiovascular disease.

Background

Adult men are more likely to die from cancer and cardiovascular disease (CVD) than women (1, 2). Various hypotheses attempt to explain this difference. It is possible that men are more susceptible to these chronic diseases, since, the overall incidence of CVD and cancer is higher in men than in women (1, 2). However, men tend to engage in more high-risk behaviors, such as smoking and alcohol consumption, than women (3). Furthermore, women utilize health care resources more frequently than men, which may prevent or forestall development and progression of diseases such as cancer and CVD (3).

Our hypothesis is that the difference in susceptibility to diseases such as cancer and CVD between males and females is due in part to fundamental physiological differences, including those that influence susceptibility to weight gain. To test this hypothesis, we studied susceptibility to obesity in male and female mice. We examined gender-specific susceptibility to obesity because it is a known risk factor for both cancer and CVD (1, 2). We conducted laboratory studies in mice to eliminate behavioral confounders that weaken research in human populations. Our results show that male mice are more susceptible to obesity than female mice, and that ovariectomy eliminates the protection against weight gain in female mice.

Methods and Procedures

At six weeks of age, C57BL/6 male, female and ovariectomized female mice (Charles River Laboratories) were randomized (15 per group) to receive one of three diet regimens: 30% calorie-restricted (CR), low-fat (5% fat) (LF), or high-fat (35% fat) (HF). A table with detailed diet formulations is found in reference 4. Briefly, the LF diet contained 19.2% protein, 67.3%

carbohydrate, and 4.3% fat; the CR diet contained 27% protein, 54% carbohydrate, and 6% fat; and the HF diet contained 26% protein, 26% carbohydrate, and 35% fat (5). The CR diet was modified so that the mice received 70% of the mean daily caloric consumption but 100% of the vitamins and minerals of the LF groups. Mice received their dietary treatments for 20 weeks. Mice were singly housed, provided with their respective diets either *ad libitum* or calorie restricted, and kept on a 12-h light/dark cycle. Food consumption was recorded twice weekly and body weight weekly. Animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin.

Ovariectomy

Surgical removal of the ovaries is a well-characterized approach to mimic the postmenopausal state in mice (5). In brief, mice were anesthetized with Avertin. Hair was clipped over the surgical area and scrubbed with Betadine and ethanol swipe. A small midline incision (~1.0 cm) was made in the skin halfway between the middle of the back and the base of the tail, starting at the last rib. The skin was moved to one side, and a small incision was made through the peritoneal lining on each side. The entire ovary was removed with a single cut between the fallopian tube and the uterine horn. The skin was then closed with a surgical staple.

Body composition

Body composition was determined after mice were sacrifice (5). Briefly, mouse carcasses were scanned using a GE Lunar Piximus Densitometer to determine percent body fat and percent lean body mass.

Statistical analyses

One-way analysis of variance (ANOVA) was used to assess the effects of diet within the various groups. We report the results of ANOVA and *a posteriori* comparison of the means using

Tukey's Honestly Significant Difference procedure. To make comparisons within the groups, α was set to 0.05 (e.g. CR male vs. HF male; and between groups, CR male vs. CR female). Analysis was done in the final body weight and percent body fat of the mice.

Results

For twenty weeks, male, female and OVX female mice were each exposed to either a CR, LF, or HF diet. Baseline body weight for all male mice was 20 ± 0.1 grams (\pm SE); values among the various groups were not significantly different ($p > 0.05$). Final body weight for male CR mice was 20 ± 0.2 , LF 35 ± 0.8 , and HF male mice 46 ± 1.0 . Baseline average body weight for all female mice was 18 ± 0.2 grams; there were no significant differences between groups. Final body weight for female groups was: CR 18 ± 0.3 , LF 27 ± 0.7 , and HF 32 ± 1.3 . Baseline body weight for all OVX-female mice was 20 ± 0.2 grams; baseline weights were not significantly different among OVX mice. Final body weight for OVX-female groups was: CR 22 ± 0.4 , LF 32 ± 1.0 , and HF 47 ± 2.1 .

Fig 1A shows that all three male groups had greater propensity to weight gain and obesity than the female groups ($p < 0.05$). Moreover, male mice consuming the LF diet developed body weights similar to those of female mice consuming the HF diet ($p > 0.05$). Differences in body weight between male and female mice were also reflected in differences in body fat levels (Fig 1B), with male mice having more body fat in all three diet categories than female mice ($p < 0.05$). To determine the effect of ovarian hormones on susceptibility to obesity in female mice, ovaries were surgically removed from 45 additional female mice, then divided into groups of 15 mice and given the three diets described above. Fig 1A shows that ovariectomized female mice

patterned like male mice in their susceptibility to weight gain. Results also show that body fat levels in ovariectomized female mice were almost identical to those of male mice (Fig 1B).

With respect to lean mass, values tended to be lower in male and OVX-female mice and higher in female mice. Lean body mass for male mice was (percent \pm SE): CR 73 \pm 1.0, LF 59 \pm 1.9, and HF 42 \pm 1.6; for female mice: CR 77 \pm 1.4, LF 69 \pm 1.7, and HF 56 \pm 1.5; and for OVX-female mice: CR 71 \pm 1.2, LF 55 \pm 2.3, and HF 38 \pm 3.6.

Discussion

Review of the literature shows that a direct comparison in susceptibility to body weight gain between males, females, and ovariectomized females has not been made. Our studies show that male mice are more susceptible to obesity than female mice, and that ovariectomy eliminates the protection of female mice from becoming obese when exposed to high-fat diets. We propose that ovarian hormones may protect females from diseases such as cancer by regulating aspects of metabolism and body composition. Evidence from animal studies supports this notion. Naugler et al. showed that male mice were more susceptible than females to liver cancer, the difference in susceptibility was eliminated by ovariectomy; and add-back of estrogen to ovariectomized female mice retrieved the protection against liver cancer (6). Moreover, we showed that male mice have a higher susceptibility of becoming insulin resistant and developing tumors than female mice, and that ovariectomy removed the protection in female mice from becoming insulin resistant and developing tumors (5). A limitation of our studies is that we did not include weight-matched male mice to the body weight of female mice. However, to include weight-matched mice, they would have to be forced to drop weight by interventions such as calorie restriction. Our unpublished observations are that calorie restricted mice over-consumed food

after they are food-restricted; thus, it is possible that the variable used to generate weight-matched mice can become a confounder. On the other hand, the initial body weight of ovariectomized female mice was similar to male mice, and their weight gain pattern was similar to male mice, suggesting that ovarian hormones indeed provide protection against weight gain.

Mouse models of obesity have helped identified new targets for the treatment of diabetes (7, 8). The model presented here may allow the identification of key signaling pathways or genes that may explain the different susceptibility to obesity between males and females. As for the relevance of this model to human biology, previously, we showed that the CR, LF and HF diets induced similar body fat phenotypes in female mice, to those found in women considered lean (BMI less than 25), overweight (BMI between 25 and 30), and obese (BMI higher than 30), respectively (9). Thus, with respect to body fat levels our diets are able to induced body fat levels (to some extent) to those found in humans. A limitation in our study is that we did not assessed body fat distribution in our mice. Moreover, it is possible that estrogen metabolism is different in mice and humans; and thus, key findings using this model would need to be verified in human samples.

In human studies, lipid profiles of pre-menopausal women are higher in HDL-C and lower in LDL-C than those of men or postmenopausal women of comparable age; however, during peri-menopausal transition, lipid profiles of women adjust to compositions similar to those of men (10). It is feasible that ovarian hormones may provide women with an additional level of protection against incipient physiological conditions such as obesity that increase susceptibility to diseases, including cancer. Numerous epidemiological studies show that obesity increases breast cancer risk only in postmenopausal women, and that the risk appears to be decreased in the presence of ovarian hormones (premenopausal phase) (11). Moreover, studies

suggest that ovarian hormone deficiency contributes to the development of CVD in women (12). On the other hand, studies such as the Women's Health Initiative (WHI) have failed to show that women randomized to the hormone replacement therapy (HRT) group had lower CVD risk than those on the placebo (13). Crucially, however, most of the women in the WHI study were already overweight or obese (12). Since our studies show that ovarian hormones protect female mice against weight gain and obesity, we suggest that HRT, given as soon as the ovaries stop producing endogenous estrogens, might decrease the risk of CVD by impeding high-risk preconditions such as obesity and insulin resistance. We recognize that endogenous and exogenous hormone may have differential effects; thus, we urge additional scrutiny to assess the roles of HRT, body-mass, and chronic disease in animal and human medical research. In particular, if women are already overweight or obese, HRT might be ineffective in preventing CVD; however, if HRT were shown to impede weight gain, then HRT would have the important secondary benefit of reducing the risk of CVD. Understanding basic metabolic differences between men and women may help us discover better treatments and preventive strategies for chronic diseases such as cancer.

Competing interests

The author(s) declare that they have no competing interests.

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Figure legend

Figure 1: Panel A. Body weight profiles for male, female and OVX female C57BL/6 mice fed CR, LF, or HF diets for 20 weeks. Male mice had a greater propensity to gain weight and become obese than female mice ($p < 0.05$). However, ovariectomy removed the female mice's protection against gaining weight, and in fact, ovariectomized female mice patterned like male mice in their susceptibility to weight gain ($p > 0.05$). **Panel B.** Percent body fat levels in male, female, and OVX-female C57BL/6 mice. Percent body fat was determined by DEXA.

Figure 1

