Abstract. Obesity is increasing worldwide. Estrogen protects female mice from gaining weight in contrast to ovariectomy. Excess weight can inhibit wound healing. We determine the effects of obesity on wound healing in the presence and absence of estrogen. For this purpose, we generated (ovariectomized (OVX) and non-ovariectomized (NOVX)) lean mice by feeding a 30% calorie-restricted diet (CR), overweight mice a low-fat (LF) diet and obese mice a high-fat (HF) diet. CR mice had the lowest, LF an intermediate, and HF mice the highest body weights. OVX exacerbated weight gain in female mice. Wounds healed fastest in CR mice regardless of estrogen status. Contrastingly, wound healing in OVX obese female mice was delayed. In sum, OVX increased the propensity of gaining weight, CR mice healed wounds more rapidly than obese mice irrespective of estrogen status, and obesity in the absence of estrogen impaired wound healing.

Obesity has become an epidemic in the United States and worldwide. It is estimated that more than 61.6% of adults are overweight or obese in the United States (2006, www.cdc.gov). In women and female animals the absence of estrogen can lead to increased weight gain (1). In rodent studies, treatment of ovariectomized (OVX) female mice with estrogen decreases the risk of gaining weight and body fat (2). Therefore, obesity in the absence of ovarian hormones may lead to greater health consequences associated with excess weight gain. In fact, we previously showed that male mice have a higher propensity to becoming obese, insulin resistant, and developing tumors compared to female mice. OVX removed the estrogen-dependent protection in female mice resulting in obesity, insulin resistance, and tumor development (3). In women, menopausal status is a risk factor for poor wound repair, as post-menopausal women exhibit a delayed healing response (4). Thus, it is likely that the effects of body weight on wound healing vary in the presence and absence of ovarian hormones. In this study, we investigated the effects of lean, overweight, and obese phenotypes on wound healing in both the presence and absence of ovarian hormones. Lean mice were generated through a calorie-restricted (CR) diet regimen, while overweight and obese mice were generated by feeding low-fat (LF) and high-fat (HF) diets, respectively. To mimic the postmenopausal phase in women we used OVX; since, removal of the ovaries leads to cessation of estrous cycles and loss of bone mineral density (5, 6). Results show that, as expected, OVX increased the susceptibility of gaining weight. Also, CR was associated with increased wound healing regardless of ovarian hormone status. However, wound healing was inhibited in OVX obese mice compared to non-ovariectomized (NOVX) obese mice.

Materials and Methods

Mouse husbandry and diets. Pathogen-free NOVX and OVX C57BL/6 female mice were purchased from Charles River Laboratories (National Cancer Institute, Frederick, MD, USA) at 6 weeks of age. They were randomized 12 mice per group to receive one of three diet regimens: 30% CR, LF (4% fat), or HF (35% fat) diets (Research Diets, Inc., New Brunswick, NJ, USA). A table with
detailed diet formulations was previously described (7). 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 group. Mice were singly housed, fed ad libitum or calorie restricted, and kept on a 12-h light/dark cycle. Food consumption was recorded twice weekly and body weight weekly. All animal protocols were approved by the Institutional Animal Care and Use Committee and conducted in compliance with guidelines established by the NIH.

Body composition. Percent body fat levels in mice were determined by scanning carcasses at the end of the study as previously described using a GE Lunar Piximus Densitometer (8).

Wound healing by secondary intention. On Day 0 of the study, mice were anesthetized with an intraperitoneal injection of a ketamine (80 mg/kg) and xylazine (10 mg/kg) mixture. The dorsum was shaved and wiped down with alcohol swabs. A dermal incision was made in the center of the dorsum using a 6mm biopsy punch, and the resulting circular piece of skin was excised, leaving a wound. A measurement of the wound length and width was done with calipers on days 0, 2, 4, 6, 8, and 10 after wounding.

Statistics. Comparisons between OVX and NOVX groups within the same diet categories were analyzed using a Student’s t-test. Comparisons between the different diet categories within either the OVX or NOVX groups were analyzed by Analysis of Variance (ANOVA) with post hoc comparison of the means using Tukey’s Honestly Significant Difference. All results are presented as the mean±standard error (SE). SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical comparisons. P-values ≤0.05 were considered statistically significant.

Results

Body weights. Figure 1A depicts the body weight profiles versus percent body fat levels of NOVX and OVX CR, LF, and HF mice after twenty three weeks on the diets. There were no significant differences in the average initial body weight among the various groups (data not shown). However, there were significant differences (p<0.0001) in average final body weight between the various groups; for NOVX: 1) CR 16.0±0.3g, 2) LF 23.5±1.1g, 3) HF 32.5±0.9g; and for OVX: 1) CR 19.5±0.4g, 2) LF 27.2±1.0g, 3) HF 38.7±1.0g. NOVX mice tended to have higher final body weights than NOVX mice (p<0.001 for CR, p<0.25 for LF, p<0.001 for HF). Figure 1B shows a representative mouse from each of the following groups: NOVX CR, LF, and HF.

Body fat levels. Average percent body fat was directly proportional to body weight measurements as shown for NOVX: 1) CR 31.6±1.8 versus LF 47.1±3.9 (p<0.0001), 2) LF 47.1±3.9 versus HF 60.2±2.4 (p<0.0016), and 3) CR 31.6±1.8 versus HF 60.2±2.4 (p<0.0095); and for OVX: 1) CR 40.4±2.3 versus LF 54.8±2.0 (p<0.0001), 2) LF 54.8±2.0 versus HF 69.8±1.5 (p<0.0001), and 3) CR 40.4±2.3 versus HF 69.8±1.5 (p<0.0001, Figure 1A).

Summing up, body fat levels tended to be higher in OVX than NOVX mice (p<0.009 for CR, p<0.113 for LF, p<0.004 for HF).

Wound healing assay. We measured the ability of NOVX and OVX CR, LF, and HF mice to heal surgically-induced wounds. Wounds were measured every two days after wounding to monitor healing progress (Figure 2A). There were no significant differences in initial wound area open among the groups (P>0.05). Results show that lean mice healed their wounds the fastest regardless of ovarian hormone status. Ten days after wounding less than 2% of the wounds remained open in the lean NOVX female mice and 5% in the lean OVX mice (p<0.622). Wound healing was similar between the LF NOVX and LF OVX female mice. Approximately 28% of the wounded area was open in the NOVX and 30% remained open in OVX LF mice at 10 days post-wounding (p<0.9001). On the other hand, obesity inhibited wound healing only in the absence of ovarian hormones. 10 days after wounding about 61% of the wound remained open in OVX obese mice compared to 34% for NOVX obese mice (p<0.002). Figure 2B shows a visual representation of the wounds at day 10 in the CR, LF, and HF OVX mice.

Discussion

The impact of diet-induced obesity and ovarian status has never been addressed concurrently with regard to wound healing. To this end, we determined the impact of obesity and estrogen status on wound healing in female mice. Results show that OVX increased the susceptibility of gaining body weight and body fat. Additionally, both obese NOVX and OVX mice displayed impaired wound healing compared to calorically restricted mice. OVX mice experienced a weight-dependent decrease in wound healing. Our results show that the susceptibility of gaining body weight is associated with poor wound healing (9, 10). On the other hand, the presence of ovarian hormones protected NOVX mice consuming the HF diet from gaining body weight. NOVX obese mice also had a better wound-healing prognosis than OVX obese mice. Our findings also show that a lean phenotype is associated with improved wound healing regardless of estrogen status.

Epidemiological evidence suggests that obesity in the absence of ovarian hormones may lead to a poorer prognosis for breast cancer, heart disease, diabetes, and neurological diseases such as Alzheimer’s (10, 11). In the present studies, we show that obesity in the absence of estrogen inhibited wound healing; however, obesity in the presence of ovarian hormones did not inhibit wound healing. It is possible that ovarian hormones protect females from these diseases by regulating aspects of metabolism and body composition.
Some animal studies support this notion. For example, Naugler et al. showed that male mice were more susceptible than females to liver cancer, and the disparity in susceptibility was eliminated by ovariectomy. Moreover, supplementing estrogen to OVX female mice reinstated the protection against liver cancer (12). Furthermore, we showed male mice have a higher susceptibility of becoming insulin resistant and developing tumors than female mice and that OVX mimicked the male condition by increasing susceptibility to insulin resistance and tumor development (3). With respect to breast cancer, obese post-menopausal women have a 50% higher risk of developing and dying from breast cancer compared to lean post-menopausal women; on the other hand, obesity before menopause does not increase breast cancer risk (13).

Understanding how obesity alters wound healing in the presence and absence of ovarian hormones may lead to better preventive or prognostic strategies for diseases such as breast cancer. Indeed, work by others suggest that gene expression profiles from wounds may be used to predict breast cancer metastasis and breast cancer patient survival (14). A possible reason wounds may help predict breast cancer outcome is due to similarities between the cancer and wound microenvironment, both are composed of intact tissue, immune cells, migrating fibroblasts, stem cells, and new blood vessels. Furthermore, both are associated with inflammation, angiogenesis, and generation of reactive oxygen species (15). Therefore, by understanding the wound-healing process in conjunction with breast cancer progression, we may be able to find surrogate markers for the early detection and prevention of breast cancer.

In summary, we show that: 1) OVX increases the susceptibility of gaining weight, 2) CR mice healed their wounds more rapidly than mice consuming LF and HF diets, irrespective of estrogen status, and 3) obesity in the absence of ovarian hormones delayed wound healing. Unraveling the mechanism by which obesity and estrogen status contribute...
to wound healing will give much needed insight toward improving wound-healing care, especially in diabetic patients, and toward potentially treating and preventing breast cancer.

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