

Research Article

Caloric Restriction Reverses Obesity-Induced Mammary Gland Inflammation in MicePriya Bhardwaj¹, Baoheng Du¹, Xi Kathy Zhou², Erika Sue¹, Michael D. Harbus¹, Domenick J. Falcone³, Dilip Giri⁴, Clifford A. Hudis⁵, Levy Kopelovich⁶, Kotha Subbaramaiah¹, and Andrew J. Dannenberg¹**Abstract**

Obesity is a risk factor for the development of hormone receptor–positive breast cancer in postmenopausal women. Estrogen synthesis is catalyzed by aromatase. Recently, we identified an obesity→inflammation→aromatase axis in mouse models and women. In mouse models of obesity, inflammatory foci characterized by crown-like structures (CLS) consisting of dead adipocytes encircled by macrophages were found in the mammary gland. CLS of the breast were found in most overweight and obese women. CLS were associated with adipocyte hypertrophy, activation of NF-κB, elevated levels of proinflammatory mediators and aromatase, and increased expression of the progesterone receptor (PR). Collectively, these findings provide a plausible explanation for the link between obesity, chronic inflammation, and postmenopausal breast cancer. Here, we investigated whether caloric restriction (CR) reversed the inflammatory state and related molecular changes in the mammary gland of obese mice. Obese ovariectomized C57BL/6J mice were subjected to 30% CR for 7 or 14 weeks. Findings in CR mice were compared with the results in mice fed a high-fat diet *ad libitum* or with control mice fed a low-fat diet. CR was associated with more than a 75% decrease in mammary CLS/cm². Reduced histologic inflammation following CR was associated with decreased adipocyte diameter and monocyte chemoattractant protein-1 (MCP-1) levels, reduced NF-κB binding activity, and normalization of levels of proinflammatory mediators, aromatase, and PR. In summary, obesity-related inflammation of the mammary gland and elevated aromatase and PR levels were reversed with CR. Our results provide a rationale for determining whether weight loss can reverse breast inflammation associated with obesity in women. *Cancer Prev Res*; 6(4); 282–9. ©2013 AACR.

Introduction

Obesity is a risk factor for the development of hormone receptor (HR)-positive breast cancer in postmenopausal women (1, 2). The development and growth of HR-positive breast cancers are commonly regulated by estrogens. Estrogen synthesis is catalyzed by aromatase, which is encoded by *CYP19* (3). Following menopause, peripheral aromatization of androgen precursors in adipose tissue is the key synthetic source of estrogen. The increased risk of developing HR-positive breast cancer in obese postmenopausal women has been attributed, in part, to increased circulating

levels of estrogen related to both excess adipose tissue and elevated aromatase expression (1, 2, 4, 5). In addition to increasing the risk of breast cancer, obesity is associated with a poor prognosis among breast cancer survivors (6–12). Obesity-related effects on hormones, adipokines, and proinflammatory mediators have been suggested to contribute to the worse prognosis of obese patients (13).

Subclinical inflammation is commonly found in visceral and subcutaneous white adipose tissue of overweight and obese women (2, 13–16). Macrophages infiltrate white adipose tissue and form characteristic crown-like structures (CLS) around dead adipocytes (15–18). These macrophages produce proinflammatory mediators (16, 19–21). Monocyte chemoattractant protein-1 (MCP-1) plays a significant role in the recruitment of macrophages to adipose tissue (22). Recently, we showed in both experimental models of obesity and obese women that CLS also occur in the white adipose tissue of the mouse mammary gland and human breast (CLS-B), respectively (23, 24). Breast inflammation, as determined by CLS-B, was paralleled by increased NF-κB-binding activity and elevated levels of proinflammatory mediators and aromatase. We concluded that the obesity→inflammation→aromatase axis may contribute to the increased risk of HR-positive breast cancer in postmenopausal women and the generally worse prognosis

Authors' Affiliations: Departments of ¹Medicine, ²Public Health, ³Pathology and Laboratory Medicine, Weill Cornell Medical College; Departments of ⁴Pathology and ⁵Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York; and ⁶Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland

Note: Supplementary data for this article are available at Cancer Prevention Research Online (<http://cancerprevres.aacrjournals.org/>).

Corresponding Author: Andrew J. Dannenberg, Department of Medicine, Weill Cornell Medical College, 525 East 68th St., Room F-206, New York, NY 10065. Phone: 212-746-4403; Fax: 212-746-4885; E-mail: ajdann@med.cornell.edu

doi: 10.1158/1940-6207.CAPR-12-0467

©2013 American Association for Cancer Research.

of obese patients with breast cancer through its impact on estrogen production. This provides a plausible explanation for the paradoxical observation that the incidence of HR-positive breast cancer rises after menopause when circulating levels of estrogen generally decline.

Decreased calorie intake is a commonly recommended lifestyle change to reduce excess adiposity and its consequences. In experimental models, caloric restriction (CR) can prolong life, reduce tumor growth and metastases, and reverse endothelial dysfunction (25–30). In the current study, we investigated whether CR could reverse histologic inflammation and related molecular changes in the mammary gland of obese mice. Our results suggest that obesity-related inflammation in the mammary gland including activation of NF- κ B, elevated levels of proinflammatory mediators and aromatase can all be attenuated by CR. These findings strengthen the rationale for evaluating whether weight loss can reverse white adipose tissue inflammation in high-risk women, which could, in turn, reduce breast cancer risk. Finally, the current results suggest that it will be worthwhile to determine whether agents with CR-mimetic properties possess antiinflammatory activity.

Materials and Methods

Materials

Lowry protein assay kits, glucose-6-phosphate, glycerol, pepstatin, leupeptin, glucose-6-phosphate dehydrogenase, and rotenone were from Sigma. 1β -[3 H]-androstenedione and [γ - 32 P]ATP were from Perkin-Elmer Life Science. Electrophoretic mobility gel shift assay kits were from Promega. RNeasy mini kits were purchased from Qiagen. MuLV reverse transcriptase, RNase inhibitor, oligo (dT)₁₆, and SYBR green PCR master mix were obtained from Applied Biosystems. Real-time PCR (RT-PCR) primers were synthesized by Sigma-Genosys.

Animal model

At 5 weeks of age, ovary intact and ovariectomized (OVX) C57BL/6J female mice (Jackson Laboratories) were randomized ($n = 9$ –14/group) to receive either low-fat (LF) or high-fat (HF) diets (Fig. 1). The low-fat (12450Bi) and high-fat (D12492i) diets contain 10 kcal% fat and 60 kcal% fat, respectively (Research Diets). The low-fat group was fed *ad libitum* for 24 weeks until sacrifice. All 4 high-fat fed groups received a minimum of 10 weeks of *ad libitum* food intake. One high-fat fed group was sacrificed after 10 weeks of *ad libitum* food intake. A second high-fat fed group was sacrificed after 24 weeks of *ad libitum* food intake. Two other high-fat fed groups received 10 weeks of high-fat feeding *ad libitum* before being 30% calorically restricted for 7 or 14 weeks, respectively. These 2 groups received 70% of the amount consumed by the high-fat group that was fed *ad libitum* for 24 weeks. To achieve this goal, *ad libitum* food intake of the high-fat group was measured daily, and on the next day, 70% of that amount was given to the 2 CR groups. Following sacrifice, mammary glands were snap frozen in liquid nitrogen and stored at -80°C for molecular analysis

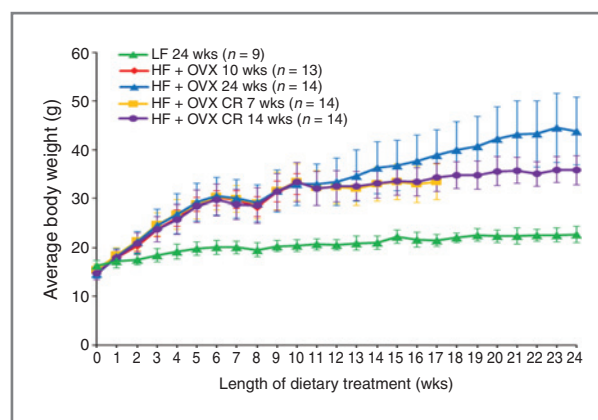


Figure 1. Caloric restriction suppresses weight gain. Body weight gain of mice in different treatment groups. The low-fat diet group was fed *ad libitum* for 24 weeks until sacrifice. All 4 high-fat fed groups received a minimum of 10 weeks of *ad libitum* food intake. One high-fat fed group was sacrificed after 10 weeks of *ad libitum* food intake. A second high-fat fed group was sacrificed after 24 weeks of *ad libitum* food intake. Two other high-fat fed groups received 10 weeks of high-fat feeding *ad libitum* before being 30% calorically restricted for 7 or 14 weeks, respectively. These 2 groups received 70% of the amount consumed by the high-fat group that was fed *ad libitum* for 24 weeks. The number of mice in each group varied as indicated from 9 to 14.

or formalin fixed for histologic analyses. The animal protocol was approved by the Institutional Animal Care and Use Committee at Weill Cornell Medical College (New York, NY).

Light microscopy

Four micron thick sections were prepared from formalin-fixed, paraffin-embedded mammary gland tissue, and stained with hematoxylin and eosin. The total number of CLS per section was quantified by a pathologist (D. Giri) and the amount of adipose tissue present on each slide was determined using NIH ImageJ. Inflammation was quantified as CLS per cm^2 of adipose tissue.

Adipocyte diameter

Adipocyte diameter was quantified as previously described (24).

Real-time PCR

Total RNA was isolated using the RNeasy Mini Kit. For tissue analyses, poly A RNA was prepared with an Oligotex mRNA Mini Kit (Qiagen). Poly A RNA was reversed transcribed using murine leukemia virus reverse transcriptase and oligo (dT)₁₆ primer. The resulting cDNA was then used for amplification. With the exception of MCP-1, primer sequences have been reported previously (23). For MCP-1, the forward and reverse primers used were 5'-AGG-TCCCTGTCATGCTTCTG-3' and 5'-GCTGCTGGTGATCC-TCTTGT-3'. Glyceraldehyde-3-phosphate dehydrogenase was used as an endogenous normalization control. RT-PCR was conducted using $2\times$ SYBR green PCR master mix on a 7500 Fast Real-time PCR system (Applied Biosystems). Relative fold induction was determined using the $\Delta\Delta C_t$ (relative quantification) analysis protocol.

Electrophoretic mobility shift assay

Nuclear extracts were prepared from mouse mammary glands using an EMSA kit (24). For binding studies, oligonucleotides containing NF- κ B sites (Active Motif) were used. The complementary oligonucleotides were annealed in 20 mmol/L Tris (pH 7.6), 50 mmol/L NaCl, 10 mmol/L MgCl₂, and 1 mmol/L dithiothreitol. The annealed oligonucleotide was phosphorylated at the 5'-end with [γ -³²P]ATP and T4 polynucleotide kinase. The binding reaction was conducted by incubating 5 μ g of nuclear protein in 20 mmol/L HEPES (pH 7.9), 10% glycerol, 300 μ g of bovine serum albumin, and 1 μ g of poly(dI-dC) in a final volume of 10 μ L for 10 minutes at 25°C. The labeled oligonucleotides were added to the reaction mixture and allowed to incubate for an additional 20 minutes at 25°C. The samples were electrophoresed on a 4% non-denaturing polyacrylamide gel. The gel was then dried and subjected to autoradiography at -80°C.

Aromatase activity

To determine aromatase activity, microsomes were prepared from tissues by differential centrifugation. Aromatase activity was quantified by measurement of the tritiated water released from 1 β -[³H]-androstenedione (31). Aromatase activity was normalized to protein concentration.

Statistics

For mouse weight at the baseline, the end of first period (10 weeks), and the end of study, differences across study groups were examined using ANOVA. Pairwise comparisons were carried out using Tukey method, which adjusts the *P* values for multiple comparisons by controlling the experiment-wise error rate. To examine mouse growth rates across study groups in each of the 2 study periods (i.e., the growth period and the intervention period), a linear mixed-effects model was used to fit the growth curves of the mice in each time period. This model takes into account between- and within-mice variation in baseline weights and growth rates. Difference in growth rates across experimental groups was examined using simultaneous tests for general linear hypotheses (32). *P* values were adjusted for multiple comparisons by controlling the false discovery rate (FDR). For the levels of various biomarkers, differences across experimental groups were examined using the nonparametric Kruskal-Wallis test. Wilcoxon rank-sum test was used to compare biomarker levels in experimental groups pairwise. *P* values were adjusted for multiple comparisons by controlling for FDR.

Results

Effects of CR on weight gain and inflammation

Initially, we investigated the effects of the different diets on the weights of mice. Treatment of ovary intact mice with a low-fat diet for 24 weeks led to a slow and gradual increase in weight (Fig. 1). To mimic the obese postmenopausal state, 4 groups of OVX mice were fed a high-fat diet for a minimum of 10 weeks. As shown in Fig. 1, a marked

increase in weight occurred in the high-fat OVX mice versus the low-fat ovary intact group. One group of high-fat OVX mice was sacrificed after 10 weeks of high-fat feeding for histologic and molecular analyses. Three other groups of high-fat OVX mice continued to be fed the high-fat diet. One group was fed *ad libitum* for an additional 14 weeks (24 weeks of high-fat feeding). This group of obese mice continued to gain weight throughout the study. The other 2 high-fat OVX groups received the high-fat diet for 10 weeks *ad libitum* followed by 30% CR (compared with mice that received the high-fat diet *ad libitum*) for either 7 or 14 weeks. In contrast to the high-fat OVX mice that received the high-fat diet *ad libitum* for 24 weeks, weight gain was suppressed in both CR groups (*P* < 0.001).

The histology of the mammary gland was assessed to quantify the severity of inflammation (Fig. 2). A typical CLS is shown in Fig. 2A. In comparison with the low-fat fed ovary intact mice, a significant increase in mammary gland inflammation (CLS/cm²) was found in the high-fat OVX mice following either 10 or 24 weeks of high-fat feeding (Fig. 2B). Notably, high-fat feeding for 10 weeks followed by CR for either 7 or 14 weeks was associated with significant decrease in the severity of mammary gland inflammation compared with the 2 high-fat fed groups. Obesity-induced adipocyte hypertrophy has been suggested to contribute to adipocyte death triggering CLS formation. Hence, adipocyte diameter was determined in each of the 5 dietary treatment groups. In comparison with the low-fat fed ovary intact mice, mammary gland adipocyte diameter was markedly increased following high-fat feeding for either 10 or 24 weeks (Fig. 2C). Following feeding a high-fat diet *ad libitum* for 10 weeks, CR for 7 or 14 weeks was associated with a small decrease in the diameter of adipocytes in the mammary gland. Because MCP-1 plays a role in the recruitment of macrophages to adipose tissue leading to CLS, levels in the mammary gland were quantified. As shown in Fig. 2D, feeding a high-fat diet to OVX mice for either 10 or 24 weeks was associated with increased levels of MCP-1, an effect that was reversed by CR for either 7 or 14 weeks.

Obesity-related induction of inflammatory mediators is attenuated by CR

Previously, activation of NF- κ B was found in the mammary glands of obese mice and the inflamed breast tissue of obese women (23, 24). Hence, we next evaluated the effects of CR on NF- κ B binding activity in the mammary gland. Feeding OVX mice a high-fat diet for either 10 or 24 weeks was associated with a marked increase in NF- κ B binding activity compared with low-fat diet fed mice (Fig. 3). Consistent with the marked improvement in histologic inflammation, CR for either 7 or 14 weeks led to a significant reduction in NF- κ B binding activity. We also quantified levels of several proinflammatory mediators [TNF- α , interleukin (IL)-1 β , and Cox-2] in the mammary gland (Fig. 4). The

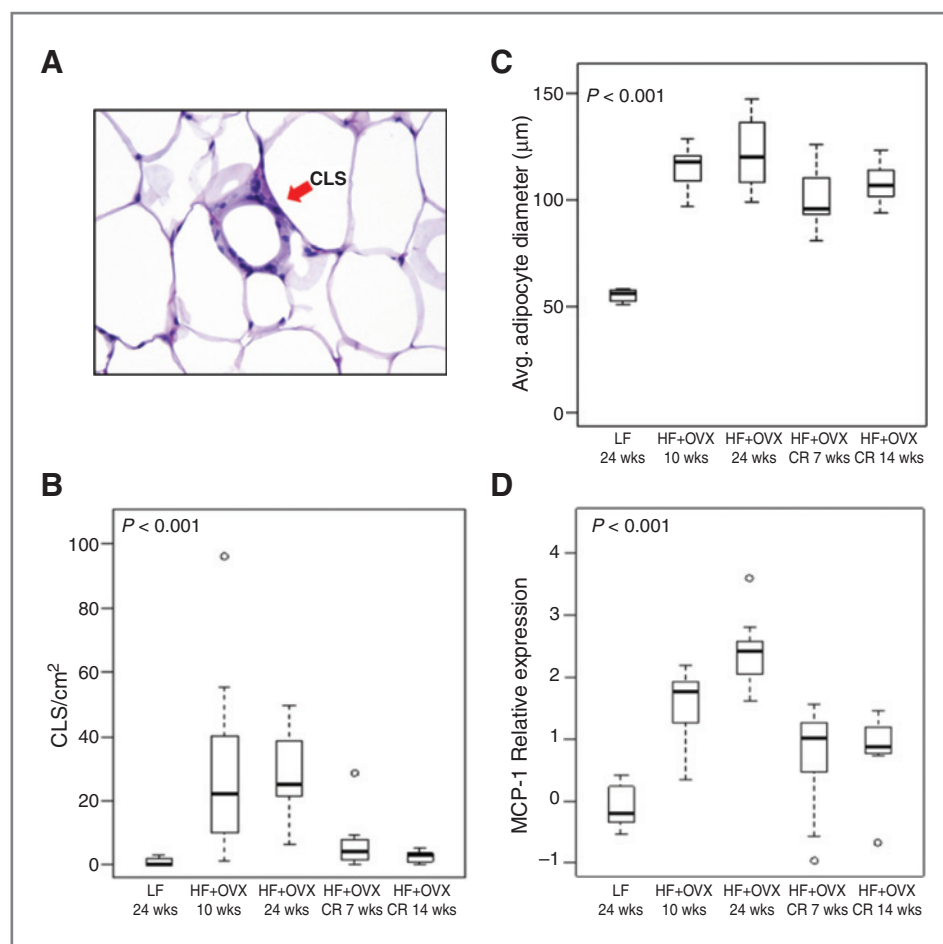


Figure 2. Mammary gland inflammation is reversed by caloric restriction. A, hematoxylin and eosin–stained section showing a CLS of the mammary gland. B, box-plot of the number of inflammatory foci per cm^2 in mammary glands of mice in the different treatment groups. Significant differences were observed across the 5 experimental groups ($P < 0.001$, Kruskal–Wallis test). In pairwise comparisons, CR for either 7 or 14 weeks led to a resolution of inflammation. Specifically, the number of CLS per cm^2 was statistically significantly decreased compared with that in mice with either 10 weeks of high-fat feeding ($P_{\text{adj}} = 0.02$ and 0.008 , respectively) or 24 weeks of high-fat feeding ($P_{\text{adj}} = 0.008$ and 0.003 respectively). C, box-plot of average mammary gland adipocyte diameter in different groups. Significant differences were observed across the 5 experimental groups ($P < 0.001$, Kruskal–Wallis test). Specifically, mammary gland adipocytes in mice with either 10 or 24 weeks of high-fat feeding were significantly larger compared with mice with 24 weeks of low-fat feeding ($P_{\text{adj}} = 0.01$ and 0.01 , respectively). CR for 7 weeks led to small yet statistically significant decrease in mammary gland adipocyte diameter compared with mice with either 10 or 24 weeks of high-fat feeding ($P_{\text{adj}} = 0.03$ and 0.02 , respectively). D, box-plot of MCP-1. Significant differences were observed across the 5 experimental groups ($P < 0.001$, Kruskal–Wallis test). Specifically, mammary gland MCP-1 levels in mice with either 10 or 24 weeks of high-fat feeding were significantly higher compared with mice with 24 weeks of low-fat feeding ($P_{\text{adj}} = 0.01$ and 0.01 , respectively). CR for 7 weeks was associated with significantly lower MCP-1 levels compared with mice in the high-fat+OVX 10 week group ($P_{\text{adj}} = 0.015$). Similarly, CR for 14 weeks was associated with significantly lower MCP-1 expression compared with mice in the high-fat+OVX 24 week group ($P_{\text{adj}} = 0.001$).

increased levels found after high-fat feeding for either 10 or 24 weeks were attenuated by CR.

Effect of CR on aromatase and PR levels

In comparison to low-fat fed ovary intact mice, increased levels of aromatase expression and activity were found in the mammary gland of OVX mice following high-fat feeding for 10 or 24 weeks (Fig. 5). CR reversed the inductive effects of the high-fat diet. Because aromatase is the rate-limiting enzyme for estrogen synthesis, we also quantified levels of *progesterone receptor (PR)*, a prototypic estrogen-inducible gene (Fig. 6). Consistent with the effects on aromatase levels, CR for either 7 or 14 weeks reversed the inductive effects of high-fat feeding on PR levels.

Discussion

Chronic inflammation of several tissues including the colon, liver, stomach, pancreas, and esophagus has been linked to an increased risk of cancer. We showed that subclinical inflammation manifested as CLS-B occurs in breast white adipose tissue of most overweight and obese women (24). CLS-B was associated with adipocyte hypertrophy, activation of NF- κ B, increased levels of proinflammatory mediators, elevated aromatase activity, and enhanced expression of PR (24, 33). Remarkably, each of these findings in human breast tissue was predicted by earlier work in a diet-induced mouse model of obesity (23). We concluded that the mouse model accurately predicts the human condition and that the

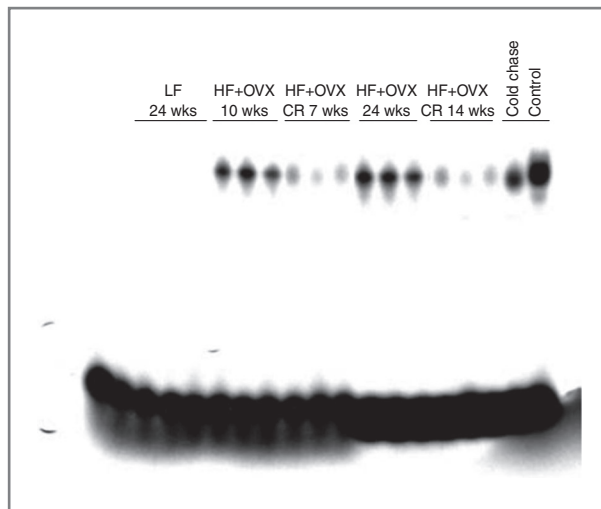


Figure 3. Obesity-related activation of NF- κ B in the mammary gland is attenuated by caloric restriction. Binding of nuclear protein from mammary glands of 15 mice (3 mice/group) in the indicated treatment groups. A total of 10 μ g of nuclear protein was incubated with a 32 P-labeled oligonucleotide containing NF- κ B binding sites. Control and cold chase, nuclear protein was incubated with labeled oligonucleotide in the absence (control) and presence (cold chase) of a 50 \times excess of cold probe. The protein-DNA complexes that formed were separated on a 4% polyacrylamide gel.

obesity \rightarrow inflammation \rightarrow aromatase axis was likely to contribute to the increased risk of HR-positive breast cancer in postmenopausal women and the worse prognosis of obese women with breast cancer.

Importantly, the availability of a mouse model, which mimics human disease, provides the opportunity to both explore the obesity \rightarrow inflammation \rightarrow aromatase axis and develop and test interventions with the goal of reversing

obesity-related inflammation of the mammary gland. In the current study, we replicated our initial findings by showing that diet-induced obesity was associated with histologic inflammation, hypertrophy of mammary adipocytes, increased NF- κ B binding activity, and elevated levels of proinflammatory mediators (TNF- α , IL-1 β , and Cox-2). Each of these proinflammatory mediators is a known inducer of aromatase (31, 34–39). Consistent with these effects, levels of aromatase mRNA, aromatase activity, and PR were all increased in the mammary glands of obese mice. In an effort to reverse this inflammatory process, we investigated the effects of CR in obese mice. Remarkably, 30% CR for either 7 or 14 weeks was associated with a marked improvement of inflammation and related molecular changes. Resolution of histologic inflammation was associated with significant reductions in NF- κ B binding activity, and reduced levels of proinflammatory mediators, aromatase, and PR. In obesity, secretion of MCP-1 by adipocytes triggers the recruitment of macrophages to adipose tissue (22). The fact that levels of MCP-1 were increased in the inflamed mammary white adipose tissue of obese mice is consistent with this concept. Moreover, the decrease in MCP-1 levels following CR provides further evidence that this factor is critical for the development of CLS because the decline was associated with reduced mammary gland inflammation.

CR prevented weight gain and led to a significant improvement in inflammation even though the decrease in mammary adipocyte size was very modest. Nonetheless, we cannot exclude the possibility that the small decrease in adipocyte size mediated by CR is important for the resolution of white adipose tissue inflammation. Previous studies have suggested that adipocyte hypertrophy predisposes to cell death and the formation of CLS (15). Hypertrophic

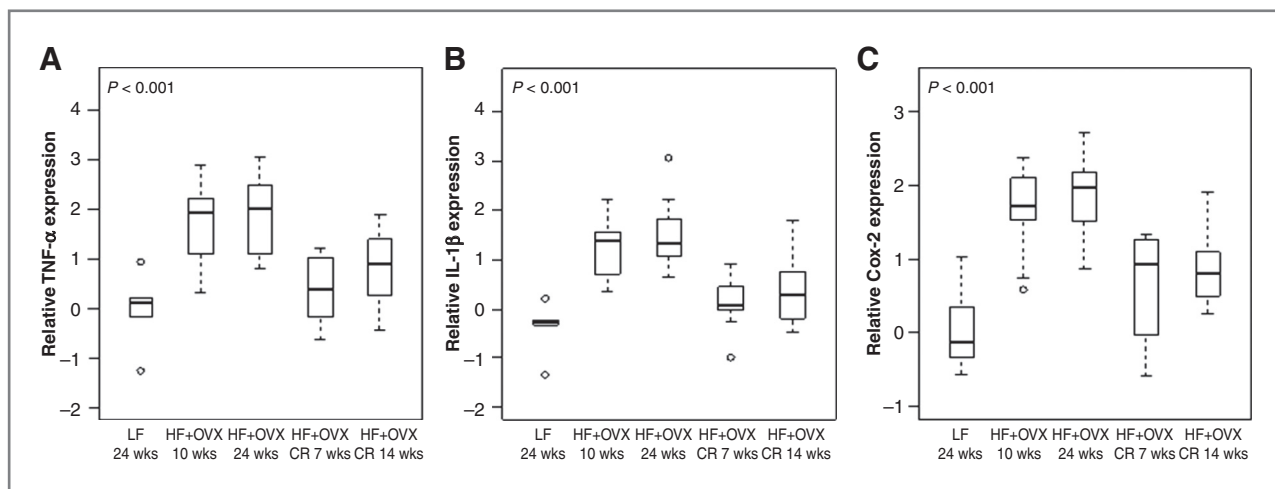


Figure 4. Obesity-related increased expression of proinflammatory mediators is reversed by CR. Real-time PCR was carried out on RNA isolated from the mammary glands ($n = 5$ –10/group) of mice in each of the 5 groups. Box-plots of TNF- α (A), IL-1 β (B), and Cox-2 (C) mRNA expression in mammary glands. Significant differences were observed across the 5 experimental groups for each proinflammatory mediator ($P < 0.001$). In pairwise comparisons, CR for 7 weeks was associated with significantly lower expression of the 3 genes in mammary gland compared with mice in the high-fat+OVX 10-week group ($P_{\text{adj}} = 0.02, 0.006, \text{ and } 0.02$, respectively). Similarly, CR for 14 weeks was associated with significantly lower expression of the 3 genes compared with mice in the high-fat+OVX 24 week group ($P_{\text{adj}} = 0.05, 0.01, \text{ and } 0.02$, respectively).

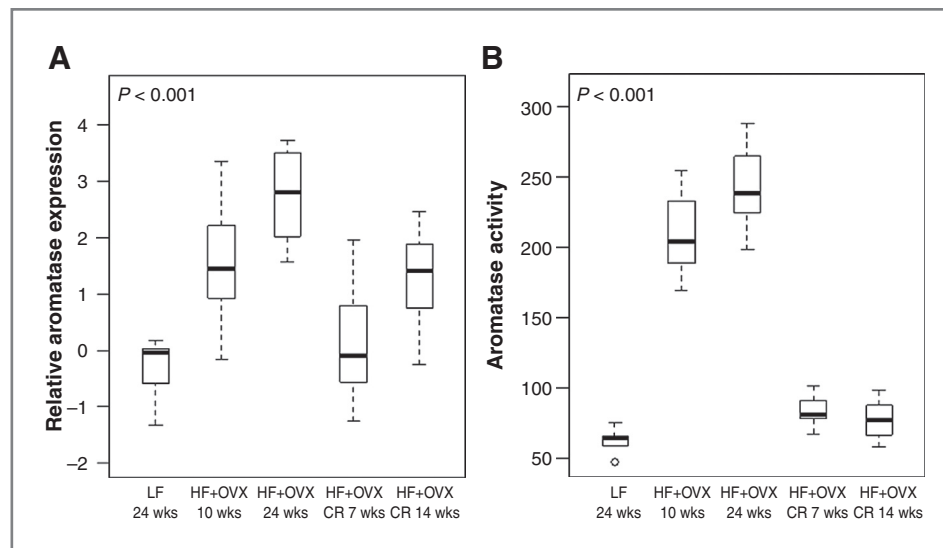


Figure 5. Caloric restriction reverses the elevated levels of aromatase found in the mammary glands of obese mice. Box-plots of relative aromatase mRNA levels (A) and activity (B) in mammary glands of mice in each of the 5 groups. Significant differences were observed across the 5 experimental groups for aromatase expression and activity ($P < 0.001$). In pairwise comparisons, caloric restriction for 7 weeks was associated with lower levels of aromatase mRNA ($P_{\text{adj}} = 0.02$) and activity (femtomoles/ μg protein/hour; $P_{\text{adj}} < 0.001$) compared with the high-fat+OVX 10-week group. CR for 14 weeks was associated with significantly lower expression of aromatase mRNA ($P_{\text{adj}} = 0.01$) and activity ($P_{\text{adj}} < 0.001$) compared with mice in the high-fat+OVX 24-week group.

adipocytes are subjected to multiple cytotoxic stressors including hypoxia, endoplasmic reticulum stress, reactive oxygen species, and free fatty acids (15, 40–42). Stress-related mechanisms of adipocyte death may be augmented

by macrophage-derived proinflammatory mediators including TNF- α . Obesity-related microcirculatory defects could also contribute to the death of hypertrophic adipocytes. In support of this possibility, treatment with a pro-tacrylin analogue that has potent vasodilating effects protected against CLS formation in high-fat diet-induced obese mice (43). On the basis of this constellation of findings, we posit that adipocytes can expand in response to energy excess until a threshold is exceeded leading to cell death and inflammation. It is possible, therefore, that CR led to a small, but physiologically significant, decrease in adipocyte size that was sufficient to protect against cell death.

Previous studies of obese humans have shown that both diet and surgically induced weight loss lead to reduced macrophage infiltration and decreased proinflammatory gene expression in subcutaneous white adipose tissue (14, 44). In addition, CR-mediated weight loss with or without exercise reduced biomarkers of inflammation in postmenopausal women (45). Although none of the previous human studies evaluated breast tissue, our findings in the murine mammary gland are certainly consistent with the results of these prior studies. The inclusion of biomarkers of inflammation such as CLS is likely to be helpful for selecting overweight or obese patients with breast cancer who are most likely to benefit from weight loss interventions. At the same time, the results of the current study raise other significant questions. It is uncertain, for example, whether endogenous or exogenous factors that suppress appetite and calorie intake will have the same beneficial effects as CR. For example, estrogen can suppress both calorie intake and inflammation (46). On the basis of our CR findings, it will be worthwhile to determine whether exogenous estrogen or other pharmacologic interventions can suppress calorie consumption and reverse the

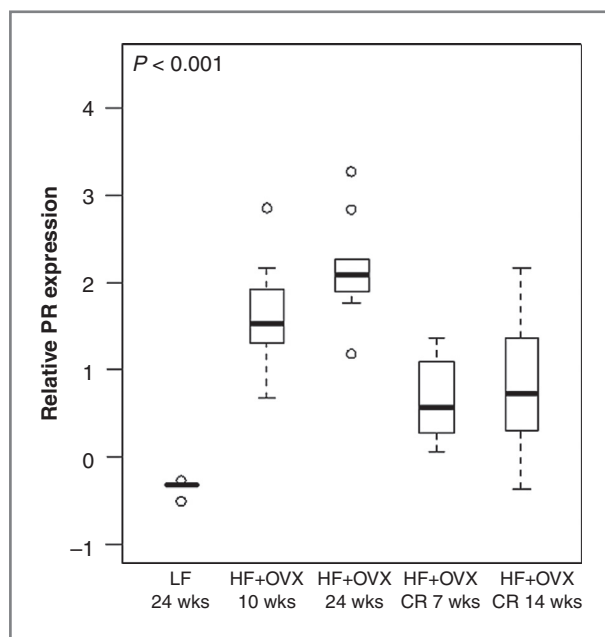


Figure 6. Obesity-related increased levels of PR in the mammary gland are reversed by caloric restriction. Box-plots of relative PR mRNA levels are shown. Significant differences were observed across the 5 experimental groups for relative PR levels ($P < 0.001$). In pairwise comparisons, CR for 7 weeks was associated with significantly lower PR levels compared with mice in the high-fat+OVX 10-week group ($P_{\text{adj}} = 0.008$). Similarly, CR for 14 weeks was associated with significantly lower PR expression compared with mice in the high-fat+OVX 24-week group ($P_{\text{adj}} = 0.007$).

obesity→inflammation axis in the mammary gland of oophorectomized mice. Ultimately, strategies that combine modest weight loss, exercise, and use of agents that mimic some of the biologic effects of CR may provide a path forward for mitigating some of the harmful effects of obesity (47).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: P. Bhardwaj, E. Sue, L. Kopelovich, K. Subbaramaiah, A.J. Dannenberg

Development of methodology: P. Bhardwaj, X. Zhou, E. Sue, K. Subbaramaiah, A.J. Dannenberg

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P. Bhardwaj, B. Du, E. Sue, M.D. Harbus, D.J. Falcone, D. Giri, K. Subbaramaiah, A.J. Dannenberg

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Zhou, D. Giri, L. Kopelovich, K. Subbaramaiah

Writing, review, and/or revision of the manuscript: P. Bhardwaj, B. Du, X. Zhou, D. Giri, C.A. Hudis, L. Kopelovich, K. Subbaramaiah, A.J. Dannenberg

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Bhardwaj, M.D. Harbus, A.J. Dannenberg

Study supervision: K. Subbaramaiah, A.J. Dannenberg

Grant Support

This work was supported by NCI 1R01CA154481, the Breast Cancer Research Foundation, and the Botwinick-Wolfensohn Foundation (in memory of Mr. and Mrs. Benjamin Botwinick).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 27, 2012; accepted February 4, 2013; published OnlineFirst February 19, 2013.

References

- Cleary MP, Grossmann ME. Minireview: obesity and breast cancer: the estrogen connection. *Endocrinology* 2009;150:2537–42.
- van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. *Cancer Epidemiol Biomarkers Prev* 2009;18:2569–78.
- Lorincz AM, Sukumar S. Molecular links between obesity and breast cancer. *Endocr Relat Cancer* 2006;13:279–92.
- Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, et al. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *J Natl Cancer Inst* 2003;95:1218–26.
- Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DE, Soderberg S, et al. Intra-adipose sex steroid metabolism and body fat distribution in idiopathic human obesity. *Clin Endocrinol* 2007;66:440–6.
- Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003;348:1625–38.
- Whiteman MK, Hillis SD, Curtis KM, McDonald JA, Wingo PA, Marchbanks PA. Body mass and mortality after breast cancer diagnosis. *Cancer Epidemiol Biomarkers Prev* 2005;14:2009–14.
- Majed B, Moreau T, Senouci K, Salmon RJ, Fourquet A, Asselain B. Is obesity an independent prognosis factor in woman breast cancer? *Breast Cancer Res Treat* 2008;111:329–42.
- Petrelli JM, Calle EE, Rodriguez C, Thun MJ. Body mass index, height, and postmenopausal breast cancer mortality in a prospective cohort of U.S. women. *Cancer Causes Control* 2002;13:325–32.
- Ewertz M, Jensen MB, Gunnarsdottir KA, Hojris I, Jakobsen EH, Nielsen D, et al. Effect of obesity on prognosis after early-stage breast cancer. *J Clin Oncol* 2011;29:25–31.
- Rose DP, Vona-Davis L. Influence of obesity on breast cancer receptor status and prognosis. *Expert Rev Anticancer Ther* 2009;9:1091–101.
- Protani M, Coory M, Martin JH. Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis. *Breast Cancer Res Treat* 2010;123:627–35.
- Hursting SD, Berger NA. Energy balance, host-related factors, and cancer progression. *J Clin Oncol* 2010;28:4058–65.
- Canello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* 2005;54:2277–86.
- Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res* 2005;46:2347–55.
- Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* 2010;72:219–46.
- Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, et al. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. *J Lipid Res* 2008;49:1562–8.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;112:1796–808.
- Suganami T, Ogawa Y. Adipose tissue macrophages: their role in adipose tissue remodeling. *J Leukoc Biol* 2010;88:33–9.
- Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003;112:1821–30.
- Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 2001;280:E745–51.
- Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 2006;116:1494–505.
- Subbaramaiah K, Howe LR, Bhardwaj P, Du B, Gravaghi C, Yantiss RK, et al. Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. *Cancer Prev Res* 2011;4:329–46.
- Morris PG, Hudis CA, Giri D, Morrow M, Falcone DJ, Zhou XK, et al. Inflammation and increased aromatase expression occur in the breast tissue of obese women with breast cancer. *Cancer Prev Res* 2011;4:1021–9.
- Ketonen J, Pilvi T, Mervaala E. Caloric restriction reverses high-fat diet-induced endothelial dysfunction and vascular superoxide production in C57Bl/6 mice. *Heart Vessels* 2010;25:254–62.
- De Lorenzo MS, Baljinnam E, Vatner DE, Abarzua P, Vatner SF, Rabson AB. Caloric restriction reduces growth of mammary tumors and metastases. *Carcinogenesis* 2011;32:1381–7.
- Hursting SD, Kari FW. The anti-carcinogenic effects of dietary restriction: mechanisms and future directions. *Mutat Res* 1999;443:235–49.
- Kritchevsky D. Colorectal cancer: the role of dietary fat and caloric restriction. *Mutat Res* 1993;290:63–70.
- Masoro EJ. Overview of caloric restriction and ageing. *Mech Ageing Dev* 2005;126:913–22.
- Fontana L, Klein S. Aging, adiposity, and calorie restriction. *JAMA* 2007;297:986–94.
- Subbaramaiah K, Howe LR, Port ER, Brogi E, Fishman J, Liu CH, et al. HER-2/neu status is a determinant of mammary aromatase activity *in vivo*: evidence for a cyclooxygenase-2-dependent mechanism. *Cancer Res* 2006;66:5504–11.

32. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J* 2008;50:346–63.
33. Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, et al. Increased levels of COX-2 and prostaglandin E2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. *Cancer Discov* 2012;2:356–65.
34. Irahara N, Miyoshi Y, Taguchi T, Tamaki Y, Noguchi S. Quantitative analysis of aromatase mRNA expression derived from various promoters (I.4, I.3, PII and I.7) and its association with expression of TNF- α , IL-6 and COX-2 mRNAs in human breast cancer. *Int J Cancer* 2006;118:1915–21.
35. Karuppu D, Kalus A, Simpson ER, Clyne C. Aromatase and prostaglandin inter-relationships in breast adipose tissue: significance for breast cancer development. *Breast Cancer Res Treat* 2002;76:103–9.
36. Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. *Endocrinology* 1996;137:5739–42.
37. Purohit A, Newman SP, Reed MJ. The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer. *Breast Cancer Res* 2002;4:65–9.
38. Hardy DB, Janowski BA, Chen CC, Mendelson CR. Progesterone receptor inhibits aromatase and inflammatory response pathways in breast cancer cells via ligand-dependent and ligand-independent mechanisms. *Mol Endocrinol* 2008;22:1812–24.
39. Salama SA, Kamel MW, Diaz-Arrastia CR, Xu X, Veenstra TD, Salih S, et al. Effect of tumor necrosis factor- α on estrogen metabolism and endometrial cells: potential physiological and pathological relevance. *J Clin Endocrinol Metab* 2009;94:285–93.
40. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010;140:883–99.
41. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004;306:457–61.
42. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004;114:1752–61.
43. Inoue E, Ichiki T, Takeda K, Matsuura H, Hashimoto T, Ikeda J, et al. Beraprost sodium, a stable prostacyclin analogue, improves insulin resistance in high-fat diet-induced obese mice. *J Endocrinol* 2012;213:285–91.
44. Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, et al. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *FASEB J* 2004;18:1657–69.
45. Imayama I, Ulrich CM, Alfano CM, Wang C, Xiao L, Wener MH, et al. Effects of a caloric restriction weight loss diet and exercise on inflammatory biomarkers in overweight/obese postmenopausal women: a randomized controlled trial. *Cancer Res* 2012;72:2314–26.
46. Musatov S, Chen W, Pfaff DW, Mobbs CV, Yang XJ, Clegg DJ, et al. Silencing of estrogen receptor alpha in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. *Proc Natl Acad Sci U S A* 2007;104:2501–6.
47. Chung JH, Manganiello V, Dyck JR. Resveratrol as a calorie restriction mimetic: therapeutic implications. *Trends Cell Biol* 2012;22:546–54.

Cancer Prevention Research

Caloric Restriction Reverses Obesity-Induced Mammary Gland Inflammation in Mice

Priya Bhardwaj, Baoheng Du, Xi Kathy Zhou, et al.

Cancer Prev Res 2013;6:282-289. Published OnlineFirst February 19, 2013.

Updated version Access the most recent version of this article at:
doi:[10.1158/1940-6207.CAPR-12-0467](https://doi.org/10.1158/1940-6207.CAPR-12-0467)

Cited articles This article cites 47 articles, 16 of which you can access for free at:
<http://cancerpreventionresearch.aacrjournals.org/content/6/4/282.full#ref-list-1>

Citing articles This article has been cited by 11 HighWire-hosted articles. Access the articles at:
<http://cancerpreventionresearch.aacrjournals.org/content/6/4/282.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerpreventionresearch.aacrjournals.org/content/6/4/282>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.