Research Article

Dietary Polyphenols Suppress Elevated Levels of Proinflammatory Mediators and Aromatase in the Mammary Gland of Obese Mice

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Abstract

In postmenopausal women, obesity is a risk factor for the development of hormone receptor–positive breast cancer driven by estrogen. After menopause, aromatization of androgen precursors in adipose tissue is a major synthetic source of estrogen. Recently, in mouse models and women, we identified an obesity–inflammation–aromatase axis. This obesity-induced inflammation is characterized by crown-like structures (CLS) consisting of dead adipocytes encircled by macrophages in breast white adipose tissue. CLS occur in association with NF-κB activation, elevated levels of proinflammatory mediators, and increased aromatase expression. Saturated fatty acids released from adipocytes have been linked to obesity-related white adipose tissue inflammation. Here we found that stearic acid, a prototypic saturated fatty acid, stimulated Akt-dependent activation of NF-κB resulting in increased levels of proinflammatory mediators [TNF-α, interleukin (IL)-1β, COX-2] in macrophages leading, in turn, to the induction of aromatase. Several polyphenols (resveratrol, curcumin, epigallocatechin gallate) blocked these inductive effects of stearic acid. Zyflamend, a widely used polyherbal preparation that contains numerous polyphenols, possessed similar suppressive effects. In a mouse model of obesity, treatment with Zyflamend suppressed levels of phospho-Akt, NF-κB binding activity, proinflammatory mediators, and aromatase in the mammary gland. Collectively, these results suggest that targeting the activation of NF-κB is a promising approach for reducing levels of proinflammatory mediators and aromatase in inflamed mouse mammary tissue. Further investigation in obese women is warranted. Cancer Prev Res; 6(9): 886–97. ©2013 AACR.

Introduction

In postmenopausal women, obesity is a risk factor for the development of hormone receptor–positive breast cancer (1, 2). In addition, obesity is associated with poor prognosis among breast cancer survivors (3–9). Estrogens commonly regulate the development and growth of hormone receptor–positive breast cancers. Aromatase, which is encoded by CYP19, catalyzes the biosynthesis of estrogen (10). After menopause, aromatization of androgen precursors in adipose tissue is the key synthetic source of estrogen. Increased circulating and tissue levels of estrogen related to both excess adipose tissue and increased aromatase levels are believed to contribute to the increased risk of hormone receptor–positive breast cancer in obese postmenopausal women (1, 2, 11, 12). Obesity-related effects on hormones, adipokines, and proinflammatory mediators have also been linked to the development and progression of breast cancer (13).

Recently, we showed in both mouse models of obesity and obese women that subclinical inflammation occurs in breast white adipose tissue (14, 15). Breast inflammation was characterized by crown-like structures (CLS) consisting of dead adipocytes surrounded by macrophages. Histologic inflammation, as determined by CLS, was paralleled by increased NF-κB binding activity and elevated levels of proinflammatory mediators and aromatase. Lipolysis is increased in obesity, which results in increased concentrations of free fatty acids (16). Saturated fatty acids trigger the activation of NF-κB in macrophages resulting in increased production of proinflammatory mediators [TNF-α, interleukin (IL)-1β, PGE2] that act, in turn, to induce aromatase in preadipocytes (14, 17). We concluded that the obesity—inflammation—aromatase axis is likely to contribute to the increased risk of hormone receptor–positive breast cancer in postmenopausal women and could similarly...
contribute to the generally worse prognosis of obese patients. In addition, to providing a plausible explanation for the paradoxical observation that the incidence of hormone receptor–positive breast cancer rises after menopause when circulating levels of estrogen generally decline, this mechanism can potentially be targeted to reduce the risk of obesity-related breast cancer.

One potential approach to reducing the risk of obesity-related breast cancer is developing strategies to suppress the activation of NF-κB and thereby reduce levels of proinflammatory mediators and aromatase. Several dietary polyphenols including resveratrol, curcumin, and epigallocatechin gallate (EGCG) have been reported to block the activation of the phosphoinositide 3-kinase (PI3K)/Akt/NF-κB pathway and inhibit carcinogenesis (18–27). Zyflamend, a widely used dietary supplement, is produced from the extracts of 10 common herbs and contains phenolic antioxidants (28–30). In preclinical models, Zyflamend has been reported to inhibit the activation of NF-κB and suppress carcinogenesis (28, 29, 31). Moreover, studies have been done to evaluate its potential to inhibit the development and progression of human prostate cancer (29, 32–34). In this study, we had 2 main objectives. First, we investigated whether Zyflamend, including pure polyphenols (resveratrol, curcumin, EGCG) contained therein, suppressed the activation of NF-κB and blocked the induction of proinflammatory mediators and aromatase in a cellular model of obesity-related inflammation. Second, we evaluated whether treatment with Zyflamend suppressed the elevated levels of proinflammatory mediators and aromatase found in the mammary glands of obese mice. Taken together, our results suggest that targeting the activation of NF-κB is a bona fide approach to suppressing levels of proinflammatory mediators and aromatase in inflamed breast adipose tissue and could provide a clinically relevant therapeutic opportunity.

Materials and Methods

Materials

Medium to grow visceral preadipocytes were purchased from ScienCell Research Laboratories. FBS was purchased from Invitrogen. Enzyme immunoassay (EIA) kits for TNF-α and IL-1β were purchased from R&D systems. Antibodies to COX-2 and β-actin were from Santa Cruz Biotechnology. Antibodies to Akt, phospho-Akt (Ser473), p65, and phospho-p65 were from Cell Signaling. Stearic acid was obtained from Amersham Biosciences. Antibodies to Akt, phospho-Akt (Ser473), p65, and phospho-p65 were from Cell Signaling.

Tissue culture

Human visceral preadipocytes (ScienCell) were grown in preadipocyte medium containing 10% FBS. THP-1 cells (American Type Culture Collection, Manassas, VA) were maintained in RPMI-1640 medium (Invitrogen) supplemented with 10% FBS. These cells were treated with phorbol 12-myristate 13-acetate (10 ng/mL) overnight to differentiate them into macrophages. THP-1 cells were then treated with vehicle, stearic acid, or stearic acid plus the indicated agent (resveratrol, curcumin, EGCG, Zyflamend). To prepare conditioned medium, these cells were treated with vehicle, stearic acid, or a combination of stearic acid plus the indicated agent for 12 hours in medium composed of RPMI-1640 and preadipocyte medium at a 1:1 ratio. Following treatment, the medium was removed and cells were washed thrice with PBS to remove stearic acid. Subsequently, fresh medium was added for 24 hours. This conditioned medium was then collected and centrifuged at 4,000 rpm for 30 minutes to remove cell debris. Conditioned medium was then used to treat preadipocytes.

Animal model

An established diet-induced obesity model was used to investigate the effects of Zyflamend on the mammary gland (14). At 5 weeks of age, ovary intact and ovariectomized (OVX) C57BL/6 female mice (Jackson Laboratories) began to receive either low- or high-fat diets. The low-fat (D12450Bi, Research Diets) and high-fat (D12492i, Research Diets) diets contain 10 and 60 kcal% fat, respectively. Ovary intact mice were fed the low-fat diet until sacrifice at 19 weeks of age and served as a lean control group. The high-fat OVX mice were fed the high-fat diet for 10 weeks (until 15 weeks of age) to induce obesity as in previous studies (14, 35). The high-fat OVX mice were then divided into 3 groups and treated with either vehicle (olive oil) or 1 of 2 doses of Zyflamend for 4 weeks while continuing to receive the high-fat diet. During the 4-week treatment phase, the mice were gavaged 5 mornings per week with 60-μL olive oil (vehicle), 20 μL (5.25 mg of extracted herbs) Zyflamend plus 40 μL olive oil, or 40 μL (10.5 mg of extracted herbs) Zyflamend plus 20 μL olive oil. The olive oil that was used as a vehicle control is the same that is found in Zyflamend. At 19 weeks of age, mice in the 4 groups were sacrificed. Following sacrifice, mammary glands were snap frozen in liquid nitrogen and stored at −80°C for molecular analysis or formalin fixed for histologic analyses. The animal protocol was approved by the
Institutional Animal Care and Use Committee at Weill Cornell Medical College.

Light microscopy

Four-μm-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 and the amount of adipose tissue present on each slide was determined using NIH Image J. Inflammation was quantified as CLS per cm² of adipose tissue.

Transient transfections

NF-κB-luciferase (Ponomics) and pSV-βgal were transfected into THP-1 cells using the Amaxa system. After 24 hours of incubation, the medium was replaced with basal medium. Subsequently, the cells were treated with vehicle, stearic acid, or stearic acid plus the indicated agent (resveratrol, curcumin, EGCG, Zyflamend, LY294002). The activities of luciferase and β-galactosidase were measured in cellular extracts.

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 using previously described primer sequences (14). Glyceraldehyde-3-phosphate dehydrogenase was used as an endogenous normalization control. RT-PCR was conducted using 2 x SYBR green PCR master mix on a 7500 Fast Real-time PCR system (Applied Biosystems). Relative fold induction was determined using the ddCT (relative quantification) analysis protocol.

Immunoblot analysis

Lysates were prepared by treating cells with lysis buffer (150 mmol/L NaCl, 100 mmol/L Tris, pH 8.0, 1% Tween 20, 50 mmol/L diethylidithiocarbamate, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL trypsin inhibitor, and 10 μg/mL leupeptin). Lysates were sonicated for 20 seconds on ice and centrifuged at 10,000 x g for 10 minutes to sediment the particulate material. The protein concentration of the supernatant was measured by the method of Lowry and colleagues (36). Total protein was extracted from snap-frozen mammary glands by homogenization using a Dounce homogenizer in 2 mL extraction buffer (25 mmol/L Tris pH 7.4, 150 mmol/L NaCl, 0.5 mmol/L sodium vanadate, 50 mmol/L sodium fluoride, 10 mmol/L sodium pyrophosphate, 1 mmol/L PMSE, 10 μg/mL trypsin inhibitor). Homogenates were centrifuged at 10,000 x g for 3 minutes at 4°C, and fat was removed from the surface by vacuum aspiration. The precipitate was resuspended by vortexing, and NP-40 was added to a final concentration of 0.5%. Samples were homogenized again using a Dounce homogenizer and centrifuged. Supernatants were decanted, and protein concentrations determined using the Coomassie protein assay. Protein solutions were stored at −80°C until use. SDS-PAGE was done under reducing conditions on 10% polyacrylamide gels. The resolved proteins were transferred onto nitrocellulose sheets and then incubated with primary antisera. Antibodies to COX-2, phospho-Akt, Akt, phospho-p65, p65, and β-actin were used. Secondary antibody to IgG conjugated to horseradish peroxidase was used. The blot was probed with the ECL Western blot detection system.

Enzyme immunoassay

TNF-α, IL-1β, and PGE₂ levels were quantified in cell culture media using EIA kits.

Electrophoretic mobility shift assay

Nuclear extracts were prepared from mouse mammary glands using an electrophoretic mobility shift assay kit. For binding studies, oligonucleotides containing NF-κB 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 μg of nuclear protein in 20 mmol/L HEPES (pH 7.9), 10% glycerol, 300 μg of bovine serum albumin, and 1 μg of poly(dI-dC) in a final volume of 10 μL 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 cell lysates and tissues by differential centrifugation. Aromatase activity was quantified by measurement of the titrated water released from 1β-[³H]-androstenedione (37). The reaction was also conducted in the presence of letrozole, a specific aromatase inhibitor, as a specificity control and without NADPH as a background control. Aromatase activity was normalized to protein concentration.

Statistics

For mouse weight data, the difference across study groups was examined using ANOVA. Tukey test was used for pairwise comparisons while adjusting for multiple comparisons. For the number of CLS per cm², the nonparametric Kruskal–Wallis test was used for comparison across study groups. Pairwise comparisons were carried out using the Wilcoxon rank-sum test. P-values were adjusted using the Holm–Bonferroni method. For proinflammatory mediators and aromatase activity data generated in the in vitro study, the nonparametric Kruskal–Wallis test was used for comparisons across experimental groups. Comparison between obese mice treated with Zyflamend (40 μL/day)
and vehicle for each biomarker was carried out using the Wilcoxon rank-sum test. For data generated in in vitro experiments, Student t test was used. All statistical tests are two-sided with significance level of 0.05.

Results

Polyphenols suppress stearic acid–mediated induction of proinflammatory mediators and aromatase

Previously, we showed that saturated fatty acids including stearic acid activated NF-kB leading to increased expression of proinflammatory mediators in macrophages including THP-1 cells (14). Here we first investigated whether several polyphenols inhibited stearic acid–mediated induction of TNF-α, IL-1β, and COX-2 in THP-1 cells. Initially, we determined if resveratrol suppressed stearic acid–mediated activation of NF-kB. As shown in Fig. 1A, 2.5 to 10 μmol/L resveratrol caused dose-dependent suppression of stearic acid–mediated induction of NF-kB luciferase activity. Based on this finding, we next investigated whether a similar dose range of resveratrol blocked stearic acid–mediated induction of proinflammatory mediators. Resveratrol in a dose-dependent fashion inhibited the induction of TNF-α, IL-1β, and COX-2 mRNA and protein (Supplementary Fig. S1A–S1C; Fig. 1B–D). Resveratrol also suppressed the increase in levels of PGE₂ in the cell culture medium of stearic acid–treated cells (Fig. 1E). TNF-α, IL-1β, and PGE₂ are all known inducers of aromatase in preadipocytes. Because resveratrol inhibited stearic acid–mediated induction of each of these proinflammatory mediators, we next determined whether it blocked the ability of macrophage-derived conditioned medium to induce aromatase in preadipocytes. Conditioned medium from stearic acid–treated THP-1 cells was a potent inducer of aromatase in preadipocytes; this inducive effect was blocked by treatment of THP-1 cells with resveratrol (Fig. 1F and G).

To determine if these suppressive properties of resveratrol were shared by other dietary polyphenols, the effects of curcumin and EGCG were investigated. Similar to resveratrol, curcumin, and EGCG blocked stearic acid–mediated activation of NF-kB (Fig. 2A and Supplementary Fig. S2A). Further, the expression of TNF-α, IL-1β, and COX-2 (Fig. 2B–D; Supplementary Figs. S1D–S1F and S2B–S2D). Levels of PGE₂ in the cell culture medium were also suppressed by both curcumin and EGCG in stearic acid–treated cells (Fig. 2E and Supplementary Fig. S2E). We also evaluated the ability of resveratrol, curcumin, EGCG, and Zyflamend on stearic acid–mediated induction of phospho-Akt. Notably, stearic acid–mediated activation of Akt was inhibited by each of the pure polyphenols (Fig. 4E–G) and Zyflamend (Fig. 4H).

Zyflamend inhibits the induction of proinflammatory mediators and aromatase

Based on the strength of these in vitro findings, we next determined if these effects could be translated in an established mouse model of obesity-associated mammary gland inflammation. Following 10 weeks of high-fat diet feeding to induce obesity, ovariectomized mice were gavaged with vehicle or Zyflamend 5 days per week for 4 weeks. Consistent with previous studies (14, 35), high-fat feeding was associated with significant weight gain. Treatment with Zyflamend did not affect either the weight of the mice (Supplementary Fig. S4) or the severity of mammary gland inflammation (Fig. 5A). However, the elevated levels of proinflammatory mediators (TNF-α, IL-1β, COX-2, PGE₂) in the inflamed mammary gland of high-fat fed mice were attenuated by treatment with Zyflamend (Fig. 5B–E). Similarly, the increased levels of aromatase mRNA and activity in the mammary glands of obese mice were partially suppressed by treatment with Zyflamend (Fig. 5F and G). To further evaluate the mechanism of action of Zyflamend, we measured levels of phospho-Akt and phospho-p65 in the inflamed mammary glands (high-fat + O VX group).
were suppressed by treatment with Zyflamend (Fig. 6A and B). Electrophoretic mobility shift assay revealed increased binding of nuclear protein to a 32P-labeled NF-κB consensus sequence in the high-fat OVX versus low-fat group, an effect that was attenuated by treatment with Zyflamend (Fig. 6C). The change in NF-κB binding activity that was mediated by treatment with Zyflamend paralleled the reduction in levels of proinflammatory mediators in the mammary gland.

Discussion

Use of either selective estrogen-receptor modulators or aromatase inhibitors reduces the risk of human receptor-positive breast cancer but the acceptance of these agents has...
been limited, in part because of concerns about toxicity (39, 40). If pathways that drive increased aromatase expression in the obese can be safely targeted, it should be possible to suppress the activation of estrogen receptor signaling, and thereby reduce the risk of hormone receptor–positive breast cancer. This would be particularly important if the active agents were well tolerated and widely available. In both a dietary model of obesity and obese women, we previously described macrophages occurring in close proximity to dead adipocytes in breast tissue, forming CLS (14, 15, 41). Activation of NF-κB and increased levels of proinflammatory mediators were paralleled by elevated levels of aromatase expression and activity in inflamed breast tissue. Analyses of the stromal-vascular and adipocyte fractions of the mammary gland suggested that macrophage-derived proinflammatory mediators induced aromatase (14). The fact that our findings in mice predicted for what was found in obese women highlights the potential relevance of the mouse model of obesity for developing risk reduction interventions.

In addition to our work on breast white adipose tissue, other studies have suggested an important role for NF-κB. Figure 2. Curcumin inhibits stearic acid–mediated induction of proinflammatory mediators in THP-1 cells. A, THP-1 cells were transfected with 1.8 μg NF-κB-luciferase and 0.2 μg pSV[β]gal. Cells were then treated with vehicle or the indicated concentration of curcumin for 2 hours. Subsequently, the cells received vehicle or 10 μmol/L stearic acid for 24 hours. Luciferase activity represents data that have been normalized to β-galactosidase. B–E, THP-1 cells were treated with vehicle or the indicated concentration of curcumin for 2 hours. Subsequently, the cells received vehicle or 10 μmol/L stearic acid for 24 hours. Enzyme immunoassay was used to measure TNF-α (B), IL-1β (C), and PGE2 (E) in the conditioned medium. Western blot analysis was used to determine levels of COX-2 protein (D). F, G, preadipocytes were incubated for 24 hours with conditioned medium from THP-1 cells that had been treated with vehicle, stearic acid (10 μmol/L), or stearic acid plus the indicated concentration of curcumin. F, real-time PCR was used to quantify aromatase mRNA; G, aromatase activity was measured in a microsomal preparation and is expressed as femtomoles/μg protein/minute. Columns, means (n = 6); bars, SD. *P < 0.01.
obesity-related white adipose tissue inflammation (42). For example, activation of NF-κB leading to elevated levels of proinflammatory mediators has been suggested to contribute to insulin resistance in obesity (42–44). A variety of polyphenols can block the activation of NF-κB (18, 19, 21, 45–47). In fact, resveratrol, curcumin, and EGCG can block the activation of the PI3K/Akt/NF-κB signaling axis (22–27). Here we reasoned that these polyphenols should block saturated fatty acid–mediated induction of proinflammatory mediators in macrophages and thereby suppress macrophage-derived conditioned medium from inducing aromatase in preadipocytes. Because of prior evidence that saturated fatty acids ranging in chain length from C12 to C18 including lauric acid, myristic acid,
Palmitic acid, and stearic acid were similar in their ability to induce proinflammatory mediators, stearic acid was used in the current experiments (14). Stearic acid activated the PI3K/Akt/NF-κB pathway in macrophages. Resveratrol, curcumin, and EGCG, 3 widely studied polyphenols, blocked stearic acid–mediated activation of NF-κB, and the induction of proinflammatory mediators in macrophages. The ability of conditioned medium from stearic acid–activated macrophages to induce aromatase in preadipocytes was also suppressed in a dose-dependent manner by treatment of the macrophages with each of these agents. These findings are in line with prior evidence that phenolic antioxidants can suppress the activation of NF-κB and reduce levels of proinflammatory mediators in mouse models of obesity (20).

Zyflamend, a polyherbal formulation that contains polyphenols including resveratrol, curcumin, and EGCG possesses anticancer properties (28–34). Given its widespread use as a dietary supplement among humans, we investigated whether Zyflamend shared some of the same effects as pure polyphenols. In fact, Zyflamend inhibited both the activation of Akt and NF-κB and the induction of proinflammatory mediators in macrophages. It also blocked the ability of conditioned medium from stearic acid–treated macrophages to induce aromatase in preadipocytes. Consistent with these in vitro findings, treatment of obese mice with

Figure 4. Polyphenols inhibit the activation of the PI3K/Akt/NF-κB signaling axis in THP-1 cells. Cells were treated with vehicle or the indicated concentration of LY294002 for 2 hours. Subsequently, the cells received vehicle or 10 μmol/L stearic acid for 2 hours. Western blot analysis was used to determine levels of phospho-Akt and Akt (A) or phospho-p65 and p65 (C). B, cells were transfected with 1.8 μg NF-κB-luciferase and 0.2 μg p55/βgal. Cells were then treated with vehicle or the indicated concentration of LY294002 for 2 hours. Subsequently, the cells received vehicle or 10 μmol/L stearic acid for 24 hours. Luciferase activity represents data that have been normalized to β-galactosidase. D, preadipocytes were incubated for 24 hours with conditioned medium from THP-1 cells that had been treated with vehicle, stearic acid (10 μmol/L), or stearic acid plus the indicated concentration of LY294002. Aromatase activity was measured in a microsomal preparation and is expressed as femtomoles/μg protein/minute. Columns, means (n = 6); bars, SD. *P < 0.01. E–H, cells were treated with vehicle or the indicated concentration of resveratrol (E), curcumin (F), EGCG (G), or Zyflamend (H) for 2 hours. Subsequently, the cells received vehicle or 10 μmol/L stearic acid for 2 hours. Western blot analysis was used to determine levels of phospho-Akt and Akt.
Figure 5. Treatment with Zyflamend leads to reduced levels of proinflammatory mediators and aromatase in the mammary glands of obese mice. Ovary intact or ovariectomized (OVX) mice (n = 8–12/group) were fed either a low-fat diet (LF) or high-fat (HF) diet beginning at 5 weeks of age. The LF fed lean mice received the diet for 14 weeks. The HF OVX mice received the HF diet for 10 weeks to induce obesity and then were randomized to 3 treatment groups. Mice in the 3 treatment groups continued to receive the HF diet but also received vehicle (olive oil), Zyflamend (20 μL/day; 5.25 mg of extracted herbs), or Zyflamend (40 μL/day; 10.5 mg of extracted herbs) by gavage 5 days per week for 4 weeks. Real-time PCR was carried out on RNA isolated from the mammary glands of mice in each of the 4 groups. A, box plot of the number of CLS per cm² in mammary glands of mice in the different treatment groups. A significant difference in the number of CLS per cm² was observed across groups (P < 0.001; Kruskal–Wallis test). In pairwise comparisons, the number of CLS per cm² was statistically increased in the 3 groups of oophorectomized mice that received HF feeding (HF+OVX) compared with LF fed group (P(adj) < 0.01, Wilcoxon rank-sum test with Holm–Bonferroni adjustment for P values). The severity of inflammation was not reduced by treatment with either dose of Zyflamend. Box-plots of TNF-α (B), IL-1β (C), COX-2 (D), and aromatase (F) expression, PGE₂ levels (E), and aromatase activity (femtomoles/μg protein/hour; G) in mammary glands of mice in each of the groups are shown. Compared with the vehicle-treated group, obese mice treated with Zyflamend (40 μL/day) had statistically significant or almost significant decrease in the levels of proinflammatory mediators and aromatase in the mammary glands (P ≤ 0.05, Wilcoxon rank-sum test). P value of this comparison for each biomarker is shown in the plots.
Zyflamend suppressed levels of phospho-Akt, NF-κB binding activity, proinflammatory mediators, and the expression and activity of aromatase in the mammary gland. Notably, levels of proinflammatory mediators were suppressed in the absence of resolution of histologic inflammation. This implies that NF-κB activation is a critical component of the signal transduction pathway that mediates the upregulation of proinflammatory mediators and aromatase but may not be important for the development or maintenance of CLS. Our findings also imply that in addition to reducing histologic inflammation (35), targeting key components of the activated inflammatory signaling pathways may be of clinical benefit. It is possible that combining interventions that attenuate histologic inflammation with molecularly targeted therapies will be more effective than either approach used alone.

It is uncertain if Zyflamend will have similar effects in obese postmenopausal women. In this context, it should be noted that the results of a human prostate study suggest that orally administered Zyflamend may be sufficiently bioavailable to have systemic effects (33). Recently, we showed that levels of urinary PGE-M, a stable end metabolite of cyclooxygenase-derived PGE_2, were increased in obese women (48). This result fits with prior evidence that levels of COX-2 and PGE_2 are increased in inflamed white adipose tissue in obesity (14, 41). Based on the current findings, it would be worthwhile to determine whether treatment with either Zyflamend or a pure polyphenol can suppress levels of urinary PGE-M in obese women. This type of experimental approach could be used prior to considering more invasive studies to determine the effects of Zyflamend or a pure polyphenol on levels of proinflammatory mediators and aromatase in subcutaneous white adipose tissue of obese individuals. Regardless of the outcome of these types of studies, our data strongly support the goal of attempting to block the activation of NF-κB as a *bona fide* strategy for suppressing levels of proinflammatory mediators and aromatase in inflamed white adipose tissue. Agents that block the activation of NF-κB or inhibit its downstream transcriptional targets are being developed and could be useful for reducing the risk of obesity-related breast cancer (49).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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