

## Research Article

## Sulindac and Sulindac Metabolites in Nipple Aspirate Fluid and Effect on Drug Targets in a Phase I Trial

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## Abstract

Regular use of nonsteroidal anti-inflammatory drugs (NSAID) has been associated with reduced risk of breast cancer. Sulindac, a nonselective NSAID with both cyclooxygenase-2-dependent and -independent activities, is a candidate for breast chemoprevention. We conducted a phase Ib trial in 30 women at increased risk for breast cancer to evaluate the breast tissue distribution of sulindac at two dose levels (150 mg daily and 150 mg twice daily for 6 weeks), using nipple aspirate fluid (NAF) as a surrogate of breast tissue drug exposure. We also explored the effect of sulindac on drug-induced biomarkers in NAF. We show that sulindac and its metabolites partition to human breast as measured by NAF levels. Sulindac intervention did not decrease 13,14-dihydro-15-keto prostaglandin A<sub>2</sub>, a stable derivative of prostaglandin E<sub>2</sub>, in NAF, but exposure was associated with a significant trend towards higher levels of growth differentiation factor 15 in NAF in women receiving 150 mg twice daily ( $P = 0.038$ ). These results are the first to show partitioning of sulindac and metabolites to human breast tissue and the first evidence for a potential dose-dependent effect of sulindac on growth differentiation factor 15 levels in NAF. *Cancer Prev Res*; 3(1); 101–7. ©2010 AACR.

## Introduction

There is considerable variation in the effect of regular use of nonsteroidal anti-inflammatory drugs (NSAID) and risk of breast cancer (1–4), although a recent meta-analysis supports a statistically significant, but modest, 12% risk reduction overall with NSAID use (5). Although the overall magnitude of benefit of any NSAID is arguably modest, the actual benefit is likely underestimated as drug duration, dose, type of NSAID, and possibly tumor subtype-specific effects are poorly captured in the observation setting (2).

NSAIDs as chemopreventives in breast cancer are supported by work in human mammary tissues and cell culture studies in which overexpression of cyclooxygenase-2 (COX2), one of two COX targets of NSAIDs, acts early in the transition from normal breast cells to malignancy (6–8). Furthermore, COX2-associated prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production has been shown to increase aromatase activity in mammary epithelial cells (9), possibly explaining the observed benefit of NSAID use in hormone receptor-positive disease (2) and the observation of lower circulating estradiol levels among regular users of NSAIDs (10).

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The use of NSAIDs to suppress prostanoid production by inhibiting COX2 activity is not a novel concept for the prevention of epithelial cancers. Although NSAID use is now well-supported for colorectal cancer, no randomized controlled trials of NSAIDs have been conducted for the prevention of breast cancer. Extant data on the activity of commonly used NSAIDs in breast tissue following oral dosing are limited, and there are no data on the safest effective doses needed to achieve biomarker modulation in breast tissues. Accumulation in breast milk of long half-life NSAIDs (e.g., naproxen, sulindac, and piroxicam) compared with short-acting agents (e.g., ibuprofen) suggests that certain agents may be more efficacious in tissue-specific chemoprevention based on their tissue-specific dose response (11).

We conducted a phase Ib trial to evaluate the distribution of sulindac and its metabolites to the breast tissue, using nipple aspirate fluid (NAF) as a surrogate of tissue exposure, at two sulindac dose levels [150 mg daily (q.d.) and 150 mg twice daily (b.i.d.)]. In addition, we explored breast tissue drug effects by measuring NAF levels of growth differentiation factor 15 (GDF15), a NSAID-induced proapoptotic protein, and 13,14-dihydro-15-keto prostaglandin A<sub>2</sub> (PGEM), a stable derivative of prostaglandins, before and after sulindac treatment.

## Materials and Methods

## Study design and eligibility

Thirty women (21–65 y) were randomized to 150 mg of sulindac q.d. or b.i.d. for 6 to 7 wk. Women were eligible if they produced  $\geq 5$   $\mu$ L of NAF at their screening visit and met

at least one of the following criteria for high risk: 5-y Gail score of >1.7% risk, medical history of lobular carcinoma *in situ* or ductal carcinoma *in situ*, at least one first-degree relative with a history of breast cancer, or *BRCA1* or *BRCA2* mutation carriage untreated with oophorectomy or mastectomy. Women with a prior history of breast cancer were eligible if they retained at least one intact, unirradiated breast and were >6 mo from all therapy including hormonal therapies. Women with any sensitivity or known contraindications to NSAID use were ineligible. Baseline NAF and blood collection were taken following a 4 to 6 wk NSAID washout. NAF and blood collection at completion of the treatment period were obtained on average within 4 h of the last sulindac dose for all subjects (range, 1-10 h). The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all participants.

### NAF collection

NAF was obtained from both breasts using self-expression with a modified breast pump under guidance of the study nurse. NAF was pooled and immediately diluted in PBS containing a protease inhibitor cocktail of AEBSF, leupeptin, DTT, and aprotinin. NAF was spun at  $200 \times g$  for 10 min; the protein-containing supernatant was aliquoted and frozen at  $-80^{\circ}\text{C}$  until analysis. The total protein concentration of each NAF sample was determined using the Micro BCA Protein Assay Kit (Thermo Scientific). The lower and upper limits of detection for the BCA were 45 and 36,000  $\mu\text{g/mL}$ , respectively.

### Blood collection and serum C-reactive protein levels

Serum was obtained from a 7 mL SST Red Top following centrifugation and stored in aliquots at  $-80^{\circ}\text{C}$  until processing for sulindac and metabolite measures. High-sensitivity C-reactive protein (hsCRP) was done by Sonora Quest Diagnostics.

### Quantification of sulindac and its metabolites in serum and NAF

Sulindac and its metabolites in serum and NAF were quantified using a high-performance liquid chromatography tandem mass spectrometry method developed in our laboratory. An aliquot of diluted serum or NAF was mixed with the internal standard solution (100 ng/mL indomethacin in acetonitrile). The mixture was acidified with 50% phosphoric acid and then extracted with a combination of ethyl acetate/hexane (1:1). The organic layer was collected and evaporated to dryness in a centrifugal evaporator. The dry residues were reconstituted with 40% acetonitrile and then injected onto the high-performance liquid chromatography mass spectrometry system.

The high-performance liquid chromatography mass spectrometry system consisted of a Surveyor HPLC system and a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Electron). Chromatographic separation of sulindac, sulindac sulfone, sulindac sulfide, and the internal standard was achieved on a Luna  $\text{C}_8$  column ( $50 \times 2$  mm,  $3 \mu$ ; Phenomenex) with a Luna  $\text{C}_8$  guard column ( $4 \times 2$  mm; Phenomenex) and a gradient of acetonitrile and water. The mass spectrometric analysis was done with the electrospray ionization interface operated in the positive polarity mode. The selected reaction monitoring transition was:  $m/z$  357  $\rightarrow$  233 for sulindac,  $m/z$  341  $\rightarrow$  233 for sulindac sulfide,  $m/z$  373  $\rightarrow$  233 for sulindac sulfone, and  $m/z$  385  $\rightarrow$  139 for the internal standard.

For each analytic run, a fresh calibration curve was prepared in the appropriate matrix and used to determine the concentrations of sulindac and its metabolites. Quality control samples were prepared and analyzed along with the authentic samples. The serum calibration curve was linear over the range of 0.33 to 167 ng/mL for sulindac and sulindac sulfide, and was in the range of 3.3 to 167 ng/mL for sulindac sulfone, with 0.15 mL aliquots of the sample volume. The NAF calibration curve was linear over the range of 0.5 to 250 ng/mL

**Table 1.** Serum concentration of sulindac and sulindac metabolites, overall and by dose

Treatment	Biomarker ( $\mu\text{g/mL}$ )	Median	Mean ( $\pm$ SD)	P*
All ( $n = 30$ )	Sulindac	2.4	2.95 $\pm$ 1.99	
	Sulfide	2.6	3.89 $\pm$ 3.55	
	Sulfone	1.6	1.95 $\pm$ 1.42	
150 mg q.d. ( $n = 15$ )	Sulindac	2.2	2.50 $\pm$ 1.93	
	Sulfide	2.4	2.43 $\pm$ 1.79	
	Sulfone	1.2	1.41 $\pm$ 0.95	
150 mg b.i.d. ( $n = 15$ )	Sulindac	3.3	3.37 $\pm$ 2.02	0.275
	Sulfide	4.0	5.35 $\pm$ 4.28	0.059
	Sulfone	1.9	2.49 $\pm$ 1.62	0.048

\*Two-sided Wilcoxon rank-sum test for difference between dose groups.

**Table 2.** Nipple aspirate concentration of sulindac and sulindac sulfide, overall and by dose

Treatment	Biomarker (ng/mL)	Median	Mean ( $\pm$ SD)	P*
All (n = 26)	Sulindac	39.8	95.8 $\pm$ 210.9	
	Sulfide	46.3	86.9 $\pm$ 121.4	
150 mg q.d. (n = 13)	Sulindac	25.9	126.7 $\pm$ 264.8	
	Sulfide	33.3	72.1 $\pm$ 106.4	
150 mg b.i.d. (n = 13)	Sulindac	39.8	65.0 $\pm$ 142.9	0.57
	Sulfide	61.9	101.7 $\pm$ 137.4	0.77

NOTE: Too few detectable values for NAF sulfone to conduct comparison between dose groups.

\*Two-sided Wilcoxon rank-sum test for difference between dose groups.

for sulindac and sulindac sulfide, and was in the range of 5 to 250 ng/mL for sulindac sulfone, with 0.1 mL aliquots of sample volume. Satisfactory results of the within-day and between-day assay precision and accuracy were obtained with both the coefficient of variation (CV) and the percentage of difference between measured and theoretical concentrations <15% over the calibration curve range.

#### PGEM as a surrogate marker of PGE2

PGEM levels were detected using the Prostaglandin E Metabolite EIA Kit (Cayman Chemicals) following validation of a modified protocol for NAF (12–14). To validate this assay for NAF, eligibility samples collected prior to randomization were pooled to determine the minimal detectable levels and to assess linear performance and between-sample and between-run variation. Standards and controls were prepared following the instructions of the manufacturer.<sup>4</sup> The lower and upper limits of detection of this assay were 1.50 and 220.5 pg/mL, respectively, for NAF. All paired samples were run in a single plate. Data were considered valid if the intra-assay CV was <5%. Ten percent of samples failed intra-assay validity and were thus reanalyzed.

#### GDF15 analysis

GDF15 content in NAF was determined using the ELISA Builder Human GDF15 DuoSet ELISA (R&D Systems), as described previously (15). To establish assay conditions for NAF and assess linearity, eligibility samples were diluted 1:20 to 1:40 at collection and run in a series of additional dilutions brought up in Reagent Diluent. The assay was conducted according to the instructions of the manufacturer. To control for interassay variation, samples from a single participant were assayed on the same plate. All samples and controls were run in duplicate. NSAID-induced and noninduced human colorectal cancer cell line

HCT116 lysates were run at two dilutions on every plate as positive controls. The lower limit of detection was 78 pg/mL. The intraplate and interplate CVs were <5% for all control samples and <8% for within-plate duplicates for the NAF samples.

#### Statistical analysis

The total number of participants randomized was 30, this constitutes the sample size for the intention-to-treat analysis. The sample size of 30 (15 per dose group) was determined to allow the detection of a standard effect size of 1.06 with 80% power based on a two-sided independent *t* test at a significance level of 5% while testing if changes differ significantly by dose. There were 15 subjects randomized to 150 mg of sulindac (Clinoril) q.d. and 15 subjects randomized to 150 mg b.i.d. for 6 to 7 wk. Values below the detectable limits of any assay were set to zero for statistical analyses. All serum samples were available for serum drug analysis. Due to technical reasons, NAF levels of drug and metabolites were not measured for four participants, resulting in a sample size of 26 (13 in each dose group) for these variables. NAF PGEM and GDF15 concentrations were adjusted by total NAF protein levels to correct for volume and dilution differences between samples. The sample size for change in NAF PGEM and GDF15 was 21 and 22, respectively, as protein levels were not detectable in all samples, which reduced the sample size in the paired analyses. The sample size for the pairs for PGEM is one fewer than for GDF15, as one NAF was exhausted before accurate measures of PGEM could be obtained. All serum samples were available for hsCRP analysis.

A two-sided Wilcoxon rank-sum test was used to test if the change in sulindac/sulindac metabolite concentration differed by dose and to test if biomarker concentrations changed from baseline to the end of the study or differed by dose. Furthermore, correlations between drug and metabolite or biomarker levels were determined using Spearman rank correlation tests. These secondary analyses were not corrected for multiple comparisons with a significance level set at 0.05.

<sup>4</sup> <http://www.caymanchem.com/analysis>

## Results

### Study participants and NAF production among eligible women

A total of 197 women were contacted for participation in the study, with 46 enrolled and 30 randomized to agent. Of the 197 contacted, 67 women refused and an additional 84 were found to be ineligible at prestudy screen. NSAID-related risk was the most common reason for ineligibility. Of the 46 women enrolled and screened for NAF production, 14 failed to produce NAF (~30%), 1 was lost to follow-up, and 1 was excluded for concomitant medicine usage not identified prior to enrollment. Each of the 30 randomized participants successfully completed the study and provided information at the end of the study. The average age of the subjects was 45.2 years with no significant differences for menopausal status between the two treatment groups (data not shown). Baseline and end-of-study paired NAF protein levels were strongly correlated ( $\rho = 0.65$ ,  $P = 0.0001$ ). After correcting for NAF dilution, there were no significant differences in the mean or median total protein values before and after sulindac treatment (59.2 versus 60.1 mg/mL and 40.3 versus 37.8 mg/mL, respectively).

### Serum levels of sulindac and metabolites

The mean and median serum levels of sulindac and each of the metabolites at the end of the study are shown in Table 1. All three compounds were detectable in the serum of all subjects ( $n = 30$ ) after sulindac treatment for 6 weeks. The mean serum sulindac, sulindac sulfide, and sulindac sulfone concentrations were 2.95,

3.89, and 1.95  $\mu\text{g/mL}$ , respectively. Dosing at 150 mg b.i.d. resulted in an average of 35%, 120%, and 77% increase in serum sulindac, sulindac sulfide, and sulindac sulfone concentrations, respectively. Serum sulfone concentrations were significantly higher in the higher dose group ( $P = 0.05$ ), whereas serum sulfide levels did not significantly differ by dose group ( $P = 0.06$ ). The levels of sulindac in serum were strongly correlated with the levels of sulfide ( $\rho = 0.83$ ,  $P < 0.0001$ ) and less strongly with the levels of sulfone ( $\rho = 0.46$ ,  $P = 0.01$ ) in serum.

### Nipple aspirate levels of sulindac and metabolites

Of the NAF samples, 26 pairs (before and after drug administration) were evaluated for levels of sulindac and its metabolites. Mean and median NAF levels of the parent compound and each of the metabolites at the end of the study are shown in Table 2. The NAF drug and metabolite concentrations ranged from undetectable to 1,157.0, 340.1, and 157.0 ng/mL for sulindac, sulindac sulfide, and sulindac sulfone, respectively, after 6 weeks of sulindac treatment. NAF sulindac, sulfide, and sulfone levels were undetectable in 10 of 26, 11 of 26, and 23 of 26 of the samples following drug administration, respectively. Six of the 10 NAF samples for which sulindac and sulfide were undetectable also had low or undetectable protein levels, suggesting that the samples may have been overly dilute to detect the drug. The mean (and median) NAF sulindac and sulindac sulfide concentrations were 95.8  $\mu\text{g/mL}$  (39.8) and 86.9  $\mu\text{g/mL}$  (46.3), respectively. For NAF sulindac sulfone, there were too few samples with detectable levels to be informative. There were no statistically

**Table 3.** Nipple aspirate concentration of PGEM and GDF15, before and after sulindac treatment, and change (post minus pre) by dose

	Pre	Post	Change (all)	Change (150 mg q.d.)	Change (150 mg b.i.d.)
PGEM (ng/mg protein)					
$n^*$	21	21	21	10	11
Median	10.5	11.2	-0.03	-0.06	0
Range	0, 200	0, 200	-64.6, +25.2	-64.6, +25.2	-49.9, +25.1
$P^\dagger$			0.519	0.307	0.893
$P^\ddagger$					0.48
GDF15 (ng/mg protein)					
$n^*$	22	22	22	11	11
Median	0.45	0.60	0	0	0.37
Range	0, 2.9	0, 3.3	-1.7, +1.4	-1.7, +0.4	-0.9, +1.4
$P^\dagger$			0.481	0.300	0.097
$P^\ddagger$					0.038

\*Sample size reflects paired samples with detectable protein both before and after drug administration. For PGEM, three samples before and two samples after drug administration had values below the limit of detection; for GDF15, seven NAF samples before and seven NAF samples after drug administration had levels below the limit of detection.

†Two-sided Wilcoxon signed-rank test for change between baseline and end of the study for all pairs.

significant differences between sulindac ( $P = 0.56$ ) or sulfide ( $P = 0.77$ ) levels in NAF by dose. There were no significant correlations between serum levels of sulindac, sulfide, or sulfone and NAF drug/metabolite levels (all  $P > 0.05$ ). However, similar to the strong correlation observed between serum sulindac and sulfide concentrations, there was also a significant positive correlation between NAF levels of sulindac and the sulfide metabolite ( $\rho = 0.69$ ,  $P < 0.0001$ ).

#### NAF PGEM at baseline and change with exposure to sulindac

Mean and median total NAF PGEM levels at baseline were 27.8 and 11.5 ng/mg of total protein, respectively, with a range of 0 to 198.7 ng/mg total protein. As shown in Table 3, NAF PGEM levels were nonsignificantly decreased overall with treatment ( $P = 0.519$ ). Change in PGEM did not differ by dose assignment (Fig. 1A and B). Of the directly measured drug and drug metabolite concentrations in serum and NAF, serum sulindac and its metabolites were all nonsignificantly inversely correlated with NAF PGEM levels (data not shown).

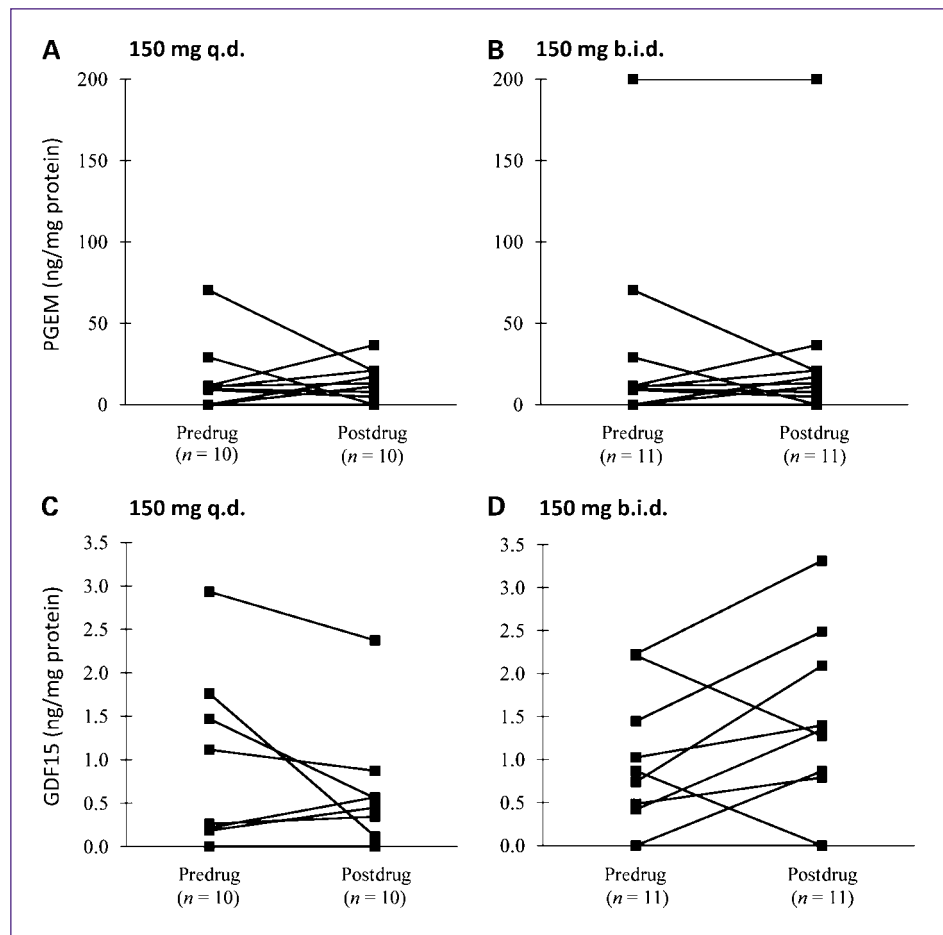
#### NAF GDF15 at baseline and change with exposure to sulindac

Mean and median total NAF GDF15 levels normalized to total protein at baseline were 0.82 and 0.45 ng/mg total protein, respectively. GDF15 concentrations at baseline ranged from 0 to 2.93 ng/mg total protein. As shown in Fig. 1C and D, NAF GDF15 levels did not change in the 150 mg q.d. dose group ( $P = 0.300$ ) but nonsignificantly increased in the 150 mg b.i.d. dose group ( $P = 0.097$ ). As shown in Table 3, this difference between the dose groups achieved significance ( $P = 0.038$ ). There were no statistically significant associations between serum levels of sulindac, sulindac sulfide, or sulindac sulfone levels and GDF15 concentrations in NAF (data not shown). Similarly, we observed no association between the concentration of sulindac in NAF or its sulfide metabolite and GDF15 levels.

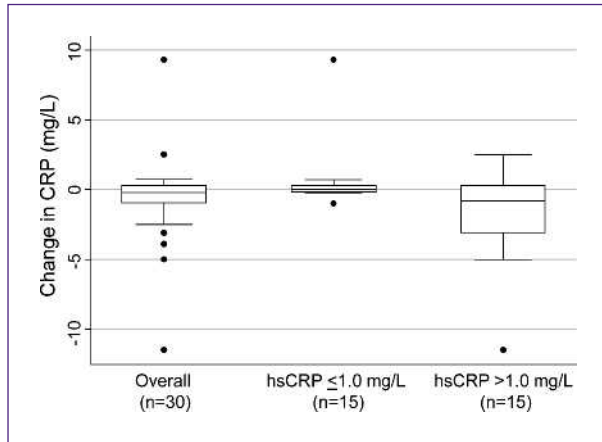
#### Serum CRP at baseline and change with exposure to sulindac

Our initial attempts to assess CRP in NAF as a recognized NSAID response biomarker and positive control biomarker failed due to the lack of a linear performance

**Fig. 1.** Change in nipple aspirate biomarker levels of PGEM and GDF15. Subject-level change in PGEM normalized by total protein for 150 mg q.d. sulindac (A) and 150 mg b.i.d. sulindac (B). Subject-level change in GDF15 normalized by total protein for 150 mg q.d. sulindac (C) and 150 mg b.i.d. sulindac (D). Note that for PGEM, 0 of 10 samples in the 150 mg q.d. dose and 3 of 11 samples in the 150 mg b.i.d. dose were undetectable (0) at baseline; whereas for GDF15, 4 of 11 samples in the 150 mg q.d. dose and 3 of 11 samples in the 150 mg b.i.d. dose at baseline were undetectable.







**Fig. 2.** Box plots showing the change in mean serum hsCRP levels for all subjects and stratified by baseline hsCRP level. Among women whose baseline CRP values were considered above normal ( $>1.0$  mg/L;  $n = 15$ ), a statistically significant decrease of 1.76 mg/L was observed ( $P = 0.02$ ).

of CRP ELISA assays for NAF samples. Western blot analyses of CRP in NAF suggest multiple uncharacterized species binding to the anti-CRP antibodies, limiting their use in this study (data not shown). As a substitute, we evaluated serum hsCRP levels as a surrogate marker of the systemic anti-inflammatory response of sulindac and used diagnostic laboratory-obtained values for hsCRP. For the entire study group, serum hsCRP levels decreased by 0.59 mg/L in response to sulindac treatment but did not reach statistical significance ( $P = 0.15$ ). Among subjects with values considered above normal at baseline ( $>1.0$  mg/L;  $n = 15$ ), a statistically significant decrease of 1.76 mg/L was observed ( $P = 0.02$ ; Fig. 2). The decrease in serum hsCRP levels was significantly greater in women who received 150 mg b.i.d. than those in the lower dose group ( $P = 0.03$ ). There were no statistically significant associations between serum levels of sulindac, sulfide, or sulfone levels and hsCRP in serum (data not shown).

## Discussion

Our study showed, for the first time, that sulindac and its sulfide metabolite can be detected in about half of the evaluated NAF samples after sulindac treatment, suggesting the partitioning of sulindac and the sulfide metabolite to human breast tissue. In contrast, the sulfone metabolite was detectable in only 3 of 26 NAF samples (11.5%). Limited studies have examined the breast tissue distribution of sulindac. Sulindac is known to be secreted in rat milk; concentrations in milk were 10% to 20% of those levels in plasma (16). Kapetanovic et al. (17) determined the plasma and mammary tissue concentrations of sulindac and sulindac sulfone in rats. The maximum plasma concentrations of sulindac and

sulfone were approximately four times higher than the maximum mammary tissue concentrations. In our study, the drug and sulfide concentrations in NAF were significantly lower than those detected in serum. The median NAF sulindac, sulfide, and sulfone concentrations were 39.8  $\mu\text{g/mL}$ , 46.3  $\mu\text{g/mL}$ , and undetectable, respectively. Compared with the average serum drug and metabolite concentrations of 2.94  $\mu\text{g/mL}$  sulindac, 3.90  $\mu\text{g/mL}$  sulfide, and 1.95  $\mu\text{g/mL}$  sulfone, the values suggest a  $\sim 100$ -fold difference between NAF and serum levels. It is likely that the breast tissue concentrations of sulindac and its metabolites are higher than those detected in NAF because the secretion of these compounds from the fatty breast tissue to NAF may be limited by their high lipophilicity (18). This suggests that in the case of sulindac, and possibly other lipophilic agents, NAF levels may not represent drug levels in the breast tissue.

Our study also explored the effects of sulindac on the putative sulindac drug targets PGEM and GDF15. These are considered exploratory end points because the study was powered only for the primary end point. We did not observe a consistent change in NAF PGEM levels with sulindac treatment. Sauter et al. (19, 20) determined the effects of celecoxib intervention at 400 mg b.i.d. or 200 mg b.i.d. on PGE<sub>2</sub> levels in women at high risk and in women with breast cancer. They showed that celecoxib at 200 mg b.i.d. taken on average for 2 weeks had no effect on NAF PGE<sub>2</sub> levels. Celecoxib at 400 mg b.i.d. significantly decreased NAF PGE<sub>2</sub> levels in postmenopausal women at high risk and in women with newly diagnosed breast cancer, but did not result in consistent changes in NAF PGE<sub>2</sub> in premenopausal women at high risk. Our study is limited by the small sample size to further stratify the analysis by menopausal status, although higher median PGEM levels were noted in premenopausal women. Collectively, these results suggest that suppression of NAF PGE<sub>2</sub> may be observed only at higher doses of COX inhibitors or under conditions with less hormonal variation.

Our study showed the presence of GDF15 in NAF and a trend for induction of GDF15 in NAF with the higher dose of sulindac. This finding is consistent with the role of GDF15 as an NSAID-inducible molecule (21) and with *in vitro* (22) and limited *in vivo* studies (23) supporting a strong drug dose-dependence on expression. These results support a direct effect of sulindac exposure on known molecular targets of sulindac in NAF.

In addition, our study assessed the effects of sulindac intervention on serum hsCRP levels. For hsCRP, we were able to show a much stronger suppressive effect of sulindac in women whose hsCRP values were above a "risk" relevant cutpoint of 1 mg/L, a cutpoint associated with cardiovascular disease (24). This finding suggests that sulindac drug effects might be detected only in individuals with abnormal expression of the biomarker of interest.

In conclusion, our results show that sulindac and its metabolites partition to human breast, as measured by NAF levels, and show trends of changes in one of the

drug effect biomarkers in NAF. Follow-up chemoprevention trials are warranted to evaluate the effects of sulindac on biomarkers of tumorigenesis in the breast tissue.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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