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## Associations between Dietary Fiber, the Fecal Microbiota and Estrogen Metabolism in Postmenopausal Women with Breast Cancer

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### Abstract

Breast cancer is a hormonally-driven cancer, and various dietary factors are associated with estrogen metabolism, including dietary fiber. Several studies report associations between dietary fiber and breast cancer; however, research on whether fiber influences circulating estrogens through the gut microbiota is rare. The objective of this cross-sectional study among 29 newly-diagnosed (stage 0-II), post-menopausal breast cancer patients is to examine associations between

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#### Authors' Contributions

**Ayse G Zengul:** 1) analysis and interpretation of data (2) revising it critically for important intellectual content; (3) final approval of the version to be published; (4) Drafting the article; (5) made substantial contributions to conception and design.

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#### Conflicts of Interest

The authors declare no conflicts of interest.

dietary fiber and the gut microbiota that are linked with  $\beta$ -glucuronidase activity, and purportedly increase circulating estrogens. Spearman's and partial correlations controlling for body mass index and age were performed using dietary recall data, Illumina MiSeq generated microbiota relative abundance, and HPLC-mass spectrometry-derived estradiol and estrone levels.

Major findings are: (1) total dietary fiber is inversely associated with *Clostridium hathewayi* ( $r = -0.419$ ;  $p = 0.024$ ); (2) soluble fiber is inversely associated with *Clostridium* ( $r = -0.11$ ;  $p = 0.02$ ); (3) insoluble fiber is positively associated with *Bacteroides uniformis* sp. ( $r = 0.382$ ;  $p = 0.041$ ); and (4) serum estradiol and estrone levels are not correlated with species/genera or dietary fiber, though there is a trend toward an inverse association between soluble fiber and estradiol levels ( $r = -0.30$ ;  $p = 0.12$ ). More studies are needed to understand the complex interaction between dietary fiber, intestinal microbiota, and hormonal levels in older females.

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## Introduction

Breast cancer affects many people worldwide. It is estimated that in 2018, approximately 266,120 US women will be diagnosed with invasive and 63,960 with noninvasive breast cancer (1). Roughly 40,920 American women will die of this disease, making it the second leading cause of cancer-related death among U.S. females (1). While breast cancer claims the lives of many women, many more are survivors. Currently, it is estimated that more than 3.1 million women are alive in the U.S. with a history of breast cancer. These women are either cancer-free or continue to live with active disease (2). Considering the threat breast cancer poses to so many women, the concern for the prevention of this disease has become forefront in today's society.

Both weight status and dietary factors appear to be associated with breast cancer risk among post-menopausal women. The mechanisms regulating estrogen production in postmenopausal women might play a role in this association (see Figure 1). Numerous studies have shown that post-menopausal obese women have a 20% to 40% increased risk of developing breast cancer compared to women of normal weight (3, 4). Each 5-unit increase in BMI is associated with a 12% increase in the risk of breast cancer in postmenopausal women (3–5). It appears that a high-fat diet, and increased circulating levels of total cholesterol and triglycerides play a role in increasing breast cancer risk (6, 7). Furthermore, high protein intake, particularly increased red meat consumption, is associated with a 13% increase in the risk of breast cancer (6, 8). On the other hand, consumption of a plant-based dietary pattern is associated with a decreased risk of breast cancer and provides protection against this prevalent disease (9).

In plant-based diets, fiber intake is prominent and associated with significantly lower breast cancer risk (10, 11). A high-fiber diet provides many health benefits. It may enhance weight loss and lower high cholesterol levels, as well as decrease insulin sensitivity (12). Considering that increased estrogen levels are associated with breast cancer development (13), the relationship between fiber and estrogen metabolism may play an important role in breast cancer prevention. It is postulated that fiber reduces circulating estrogen levels by altering the gut microbiota and decreasing deconjugation and reabsorption of estrogen. By accelerating intestinal transit and binding to estrogen in the intestine, fiber decreases serum

estrogen concentrations and prevents free hormones, such as  $17\beta$ -estradiol, to be reabsorbed (see Figure 2) (10, 14).

Further, dietary fiber may alter the gut microbiota and influence estradiol metabolism through specific enzyme activities, such as  $\beta$ -glucuronidase (14). Normally, estrogens circulate throughout the body until they reach the liver where they are inactivated through conjugation. Inactivated estrogens are then transported to the intestine for excretion into the stool. However, specific bacterial genera encode  $\beta$ -glucuronidase, which re-activates conjugated estrogens in the gut (see Figure 3). Deconjugated estrogens are reabsorbed and influence estrogen metabolism which is associated with hormone-dependent cancers, such as breast cancer (15, 16). To better understand the association between fiber intake and breast cancer, this study investigates associations between dietary fiber and the gut microbiota that promote  $\beta$ -glucuronidase activity and explores associations with estrogen levels in the blood. We hypothesize that higher levels of dietary fiber will be associated with lower abundance of intestinal microbiota that promote  $\beta$ -glucuronidase activity while lower abundance of intestinal microbiota will be significantly associated with lower levels of circulating  $17\beta$ -estradiol and estrone.

## Materials and Methods

### Experimental Plan

**Study Design**—This cross-sectional study includes baseline data from a randomized controlled trial of weight loss conducted among 29 post-menopausal women who were newly-diagnosed with stage 0–II breast cancer and treatment naïve. The detailed methods of this trial have been published previously and are summarized below (17).

**Recruitment/Consent**—Study subjects were recruited from the University of Alabama at Birmingham (UAB) Kirklin Interdisciplinary Breast Health Clinic (Birmingham, Alabama, USA). The trial was registered with the National Clinical Trials database ([NCT02224807](https://clinicaltrials.gov/ct2/show/study/NCT02224807)) and approved by the UAB Institutional Review Board (IRB-130325009).

Eligibility criteria for the study included being overweight or obese (BMI of 25–60 kg/m<sup>2</sup>) with histopathologically-confirmed stage 0–II breast cancer and scheduled for surgery as primary treatment. Patients were screened to ensure there were no preexisting medical conditions that would prevent adherence to unsupervised exercise. Also, physician clearance for any conditions, such as resting blood pressures >99 diastolic or >159 systolic, or cardiac anomaly was obtained. Participants had no current medical conditions that would affect weight status, such as Cushing's syndrome or untreated hypothyroidism, nor additional active malignancy. The participants were newly diagnosed with breast cancer, and hence had yet to begin hormonal therapy, and none of our subjects reported nephritis, enteritis, antibiotic/antimicrobial use, or the use of mitochondrial uncouplers (laxative use was not assessed). Those who met these criteria were informed about the study, provided written informed consent, and were enrolled.

**Baseline Assessment**—Study staff collected and recorded data for demographics, height/weight, medical history, and medication use (including recent use of antibiotics) (17).

A multiple-pass method was used to collect two 24-hour dietary recalls that represented normal eating habits for one weekday and one weekend day. The data were entered and analyzed using the Nutrition Data System for Research (NDSR 2014, Minneapolis, Minnesota, USA). Phlebotomy was performed after a 12-hour fast and serum was obtained. Stool samples were collected using a sterile wipe after a bowel movement prior to the baseline assessment. All samples were stored at  $-80^{\circ}\text{C}$ .

**LC-MS Analysis of  $17\beta$ -Estradiol and Estrone**—Assays for  $17\beta$ -estradiol and estrone in serum were performed in the UAB Targeted Metabolomics and Proteomics Laboratory. Estrogen analyses were determined by isotope dilution HPLC-electrospray ionization-multiple reaction ion mass spectrometry adapted from the method of Tai and Welch (18).  $17\beta$ -Estradiol and estrone standards were prepared in 0.05% BSA. Sera (500  $\mu\text{l}$ ) were diluted 1:1 with MilliQ  $\text{H}_2\text{O}$ . Samples and standards were spiked with 0.5 ng/ml  $^{13}\text{C}_6$ -estradiol (CIL, Tewksbury, MA) internal standard. Diluted samples (1 ml) and standards were loaded onto individual 30 mg Polymeric Strata-X Solid Phase Extraction cartridge columns (Phenomenex, Torrance, CA). The cartridges were washed with MilliQ  $\text{H}_2\text{O}$  (1 ml) and 40% methanol (1 ml) followed by elution of  $17\beta$ -estradiol with 1 ml of methanol. Sample eluents were dried under a gentle stream of  $\text{N}_2$ . Sodium bicarbonate (50 ml, 100 mM, pH 10.5) and dansyl-chloride (50  $\mu\text{l}$ , 1 mg/ml) in acetone were added to samples which were incubated at  $60^{\circ}\text{C}$  for 10 min. Samples were dried once more under a gentle stream of  $\text{N}_2$  followed by reconstitution in 100 ml of 40% methanol/0.1% formic acid (FA). Chromatography was carried out using an Ace Excel  $\text{C}_{18}$ -Aromatic 1.7 mm  $50 \times 3.0$  mm IS column at  $50^{\circ}\text{C}$  using a 20AD Prominence HPLC (Shimadzu, Kyoto, Japan) in tandem with 6500 Qtrap mass spectrometer (SCIEX, Framingham, MA). LC-MS operation and data collection were under the control of Analyst 1.6.2 software (SCIEX). The mobile phases were composed of (A) 0.1% FA and (B) acetonitrile 0.1% FA; the flow rate was 300  $\mu\text{l}/\text{min}$ . Gradient starting conditions were 50% B which was held for 1 min, a linear increase of B to 100% B at 4 min, held at 100% B until 4.75 min, and returned to 50% B at 5 mins, to equilibrate to starting conditions until 7 min. LC flow was diverted to waste for the first 1.8 min to prevent salt contaminating the MS front end. The MS was operated in positive electrospray ionization mode with the following parameters: curtain gas 30, collision gas medium, temperature 500, ion spray voltage 5000, collision energy 25, GS1 60 and GS2 60. Mass transitions for multiple-reaction-monitoring mode were  $m/z$  506/171 for dansyl- $17\beta$ -estradiol, 504/171 for dansyl-estrone and  $m/z$  512/171 for  $^{13}\text{C}_6$ -dansyl- $17\beta$ -estradiol. The standard curve ranged from 5 – 5000 pg/ml over seven points. All data were processed, and concentration factors were corrected using Multiquant 3.0.1 (SCIEX).

**Fecal Microbe Analysis**—Stool samples were collected by participants after defecation using a sterile wipe, placed in a plastic bag, and kept in their home freezer until baseline assessment, at which time the samples were collected and stored at  $-80^{\circ}\text{C}$  until analyzed.

Fecal DNA extraction was carried-out using Zymo Fecal DNA Miniprep kit. Microbiome analysis targeting the V4 region of the 16S rRNA gene was performed using an Illumina MiSeq (19). The post DNA sequence analysis used Quantitative Insight into Microbial

Ecology (QIIME) suite, V.1.7 with modifications as described in Kumar et al. (19) and Fruge et al. (20, 21).

**Variables and Statistical Analyses**—This study was a secondary analysis that utilized data on 29 post-menopausal women. The outcome variables of interest were dietary intake of fiber, serum 17 $\beta$ -estradiol, BMI, and the bacterial genera and species that colonize the human intestinal tract that encode for  $\beta$ -glucuronidase based on the findings of Kwa et al. (15).

Normality tests for all variables of interest were assessed. Due to non-normal distribution, the Spearman rank test was utilized to reveal any potential associations between gut microbiota, 17 $\beta$ -estradiol, estrone, and dietary fiber (total, soluble and insoluble). In addition to the individual correlations between gut microbiota at the genera and species levels, the microbiota linked at phylum, family and class levels were combined, and the correlations between these, 17 $\beta$ -estradiol, estrone and the dietary fiber types were explored. Further analyses, including partial correlation tests between the dietary fiber types and specific microbiota, were performed by controlling for BMI and age. Control for the presence of diabetes was considered, but because no significant differences were found in the microbiota among participants with this comorbidity vs. those without, it was not used as a covariate. All analyses were performed using IBM SPSS (version 24.0).

## Results

A total of 32 women enrolled in the study and provided fasting blood and fecal samples. We excluded premenopausal ( $n = 2$ ) and perimenopausal ( $n = 1$ ) women because fluctuating hormone levels affect circulating estrogen concentrations. Characteristics of participants are provided in Table 1. The study participants had a mean age of 62.4 years and 82.1% were obese. Most were Non-Hispanic White or African-American, the remainder were of mixed race. Most were recently diagnosed with invasive cancers that were both estrogen- and progesterone-positive, though none had begun hormonal therapy. Other medications found to affect the microflora, eg., antibiotics/antimicrobials and mitochondrial uncouplers were not reported by any participants.

Table 2 presents data on dietary fiber, microbiota, estradiol concentrations, and the relative abundance of investigated species and genera. Total dietary fiber intake was roughly 14 g/day of which insoluble fiber comprised the major proportion. The relative abundance values of each phylum for each subject are presented in Figure 4. The most common phyla were *Firmicutes* and *Bacteroidetes*. At the genus and species levels, a higher abundance of *Faecalibacterium* and a lower abundance of *Bifidobacterium*, *Clostridium-perfringens*, and *Clostridium hathewayi* were detected in the samples (Table 2).

Correlations between dietary fiber types, gut microbiota, 17 $\beta$ -estradiol and estrone concentrations are displayed in Table 3. The results indicate that total dietary fiber intake is significantly and inversely associated with *Clostridium hathewayi* sp. ( $r = -0.419$ ;  $p = 0.024$ ). While the strength of association was somewhat weaker, soluble dietary fiber was significantly and inversely associated with *Clostridium* ( $r = -0.11$ ;  $p = 0.02$ ). Both of these

inverse relationships continued to be observed after controlling for age and BMI. Also, insoluble dietary fiber was significantly and positively associated with *Bacteroides uniformis* sp. ( $r = 0.382$ ;  $p = 0.041$ ), again an association that remained after controlling for age and BMI. There were marginally significant correlations between certain gut microbiota and dietary fiber types (Table 3). For example, the association between insoluble dietary fiber and *Clostridium hathewayi* sp. was marginally significant ( $r = -0.31$   $p = 0.066$ ). There also were marginally significant positive associations between *Escherichia coli* sp., *Escherichia* and total dietary fiber ( $r = 0.35$ ;  $p = 0.059$ ).

Relatedly, there was a trend toward an inverse association between soluble dietary fiber and  $17\beta$ -estradiol levels ( $r = -0.30$ ;  $p = 0.12$ ), albeit statistically significant. Serum  $17\beta$ -estradiol and estrone levels were not correlated either with species/genera nor with dietary fiber types. Moreover, combined genera and species at the phylum, family and class levels and the summative proportion of the microbes at the genus and species levels did not reveal any significant associations.

## Discussion

Our study is one of the few to examine the relationship between the gut microbiota that promote  $\beta$ -glucuronidase activity, dietary fiber and circulating  $17\beta$ -estradiol and estrone. Contrary to our hypothesis, the results of this study indicate that dietary fiber intake had no relationship to estrogen levels in the blood. However, we found that higher levels of total and soluble dietary fibers correlate with lower levels of *Clostridium hathewayi* sp. and *Clostridium* (*Erysipelotrichaceae* family), respectively. These bacteria promote  $\beta$ -glucuronidase activity and the inverse relationship between them and dietary fiber continued to be observed after controlling for age and BMI. Contrary to these findings, we also found a positive and significant relationship between insoluble fiber and *Bacteroides uniformis* which also promotes  $\beta$ -glucuronidase activity. Given the important role of  $\beta$ -glucuronidase enzyme in luminal hormone metabolism, this exploratory analysis provides valuable insights for future studies.

To date, no studies have found a link between *Clostridium hathewayi* and dietary fiber. However, our results demonstrated the significant inverse relationship between total dietary fiber intake and *Clostridium hathewayi*, a newly discovered *Clostridium* species (22), which has been implicated in clinical diseases, such as sepsis and infection (23–27). Thus, the association between total dietary fiber and *Clostridium hathewayi* may have important implications regarding the prevention of both infectious diseases, as well as those that are hormonally driven.

Another inverse relationship observed was between soluble dietary fiber and *Clostridium*. However, this result is not in accordance with findings reported by Chinda et al. (28) and Bang et al. (29) who reported that pectin, which is a soluble fiber, was associated with higher levels of *Clostridium*. These studies were conducted exclusively in healthy males, unlike our study which included female postmenopausal breast cancer patients, and included far fewer study participants, i.e.,  $n = 14$  and  $n = 3$ , respectively. Also, our outcome variables included total soluble dietary fiber, rather than pectin alone. Because *Clostridium* produces

the short chain fatty acids acetate and butyrate it might be important for enhancing intestinal peristalsis and in preventing/treating constipation, diarrhea, and colitis (29). Therefore, further research is warranted to clarify the relationship between dietary fiber and *Clostridium* and elucidate specific mechanistic relationships.

Among the three dietary fiber types, the only significant positive relationship we found was between insoluble fiber and *Bacteroides uniformis*. Higher levels of insoluble fiber were associated with higher levels of *Bacteroides uniformis*. This finding may relate to the influence of *Bacteroides uniformis* on glycolysis pathways. According to a study that was conducted by Benítez-Páez on 75 full-term newborns, “*B. uniformis* strains exhibit an expanded glycolytic capability when compared with other *Bacteroides* species” (30). Enhanced glycolysis is related to higher glucose uptake, and it is currently used as an indicator of malignancy since it represents an evident characteristic of many cancers (31, 32). Considering the relationship between *Bacteroides uniformis* and glycolysis, our results, thereby, might shed light on understanding the influence of *Bacteroides uniformis* on glycolysis and its possible physiological effects in breast cancer.

Although we found some significant associations, we did not find support for our hypothesis regarding the relationship between dietary fiber and levels of circulating estrogens. We found only a weak inverse association between soluble fiber and  $17\beta$ -estradiol. This result is consistent with the findings of Gaskins et al. (2009) who observed a significant and inverse relationship between dietary fiber consumption and  $17\beta$ -estradiol concentrations among 250 premenopausal healthy women (aged 18–44 y) (13). In this much larger prospective cohort study, both soluble and insoluble fiber had an inverse relationship with  $17\beta$ -estradiol concentrations ( $\beta = -0.222, p = 0.01$ ;  $\beta = -0.057, p = 0.02$ , respectively) (13). Despite highly fluctuating  $17\beta$ -estradiol levels among premenopausal women, the significant associations they found suggest that if this relationship was explored among larger samples of postmenopausal women, significant results might be attained.

Ours is the first study to correlate dietary constituents and  $17\beta$ -estradiol/estrone levels, and to explore their relationship with gut microbiota involved with  $\beta$ -glucuronidase activity among post-menopausal women with breast cancer. However, it is important to recognize study limitations, which include a small sample size and cross-sectional design that resulted in a limited ability to detect associations and reduced the statistical power. Although we adjusted for BMI and age, our results could still have been affected by residual or unmeasured confounding; for example, we did not collect data on laxative use which may have affected the results. The range of  $17\beta$ -estradiol and estrone levels in post-menopausal women, for example, is narrow and levels are low - thus the distribution may not have been sufficient to detect correlations. Moreover, because our sample was fairly circumscribed, ie., post-menopausal breast cancer patients, our results may not generalize to other populations, such as females without breast cancer and males. Finally, multiple testing and the chance of uncovering associations that are spurious is a limitation of this study. Despite these limitations, this study makes a unique contribution, first because very few studies have investigated microbiota among populations with breast cancer, and second, this study relies on dietary data that includes soluble, insoluble, and total fiber.

## Conclusion

The role of bacterial  $\beta$ -glucuronidase activity in breast cancer risk is still obscure. The influence of dietary fiber on this activity has an important bearing on understanding the link between estrogen metabolism and breast cancer. By being one of the few studies investigating the triad of these overlapping relationships among dietary fiber,  $\beta$ -glucuronidase activity and serum estrogens, our study provides insight and direction for future studies.

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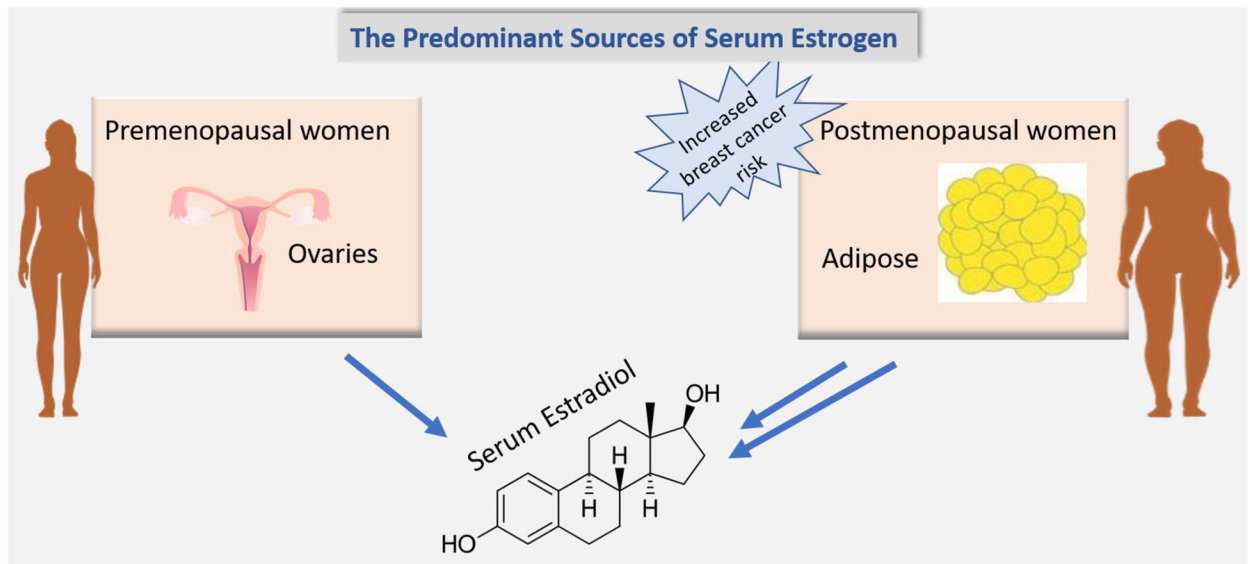
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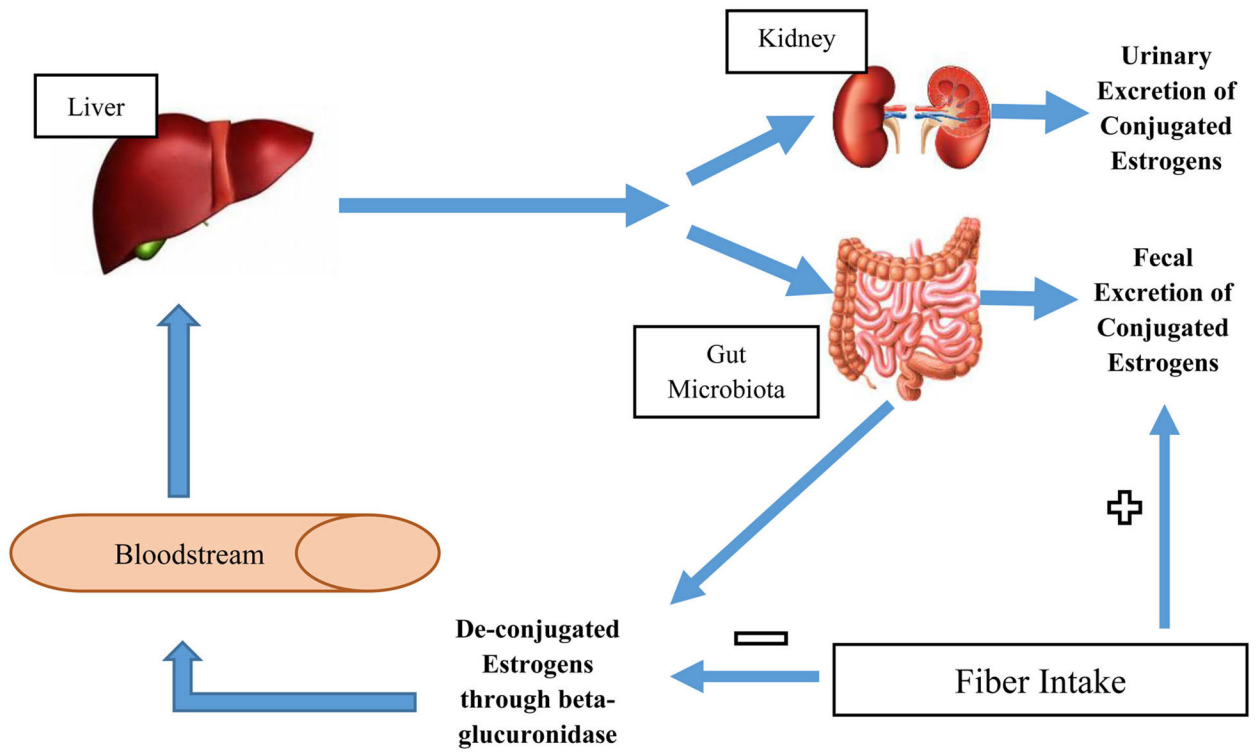
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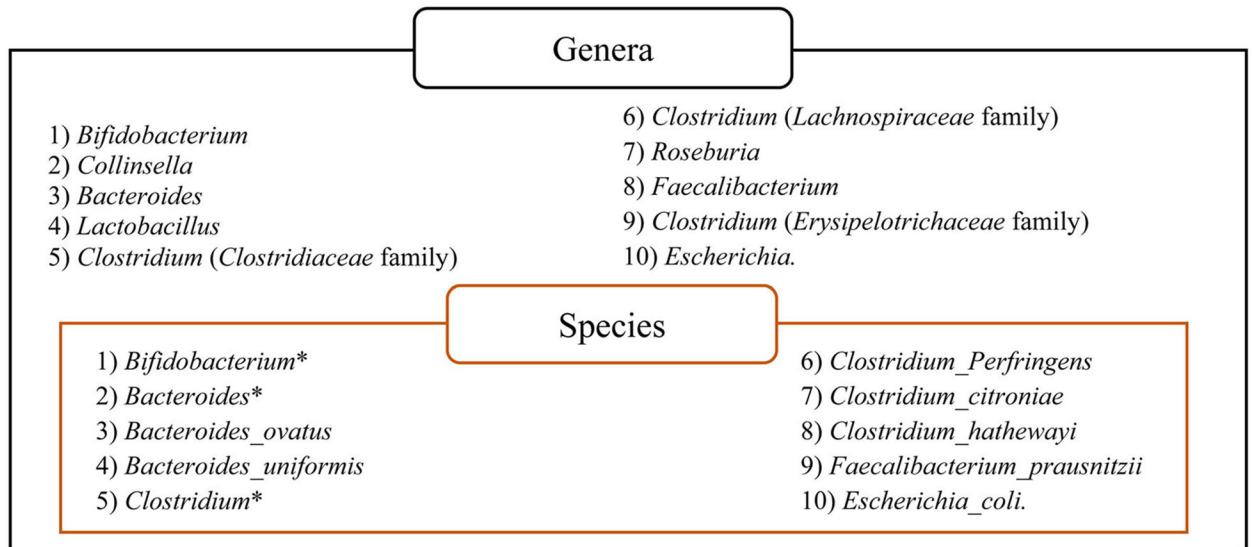


**Figure 1.**

A framework indicating sites of conversion of androgen to estrogen. In premenopausal women, the ovaries are the predominant source of serum estradiol; however, in postmenopausal women, estradiol mainly comes from aromatization of adrenal and ovarian androgens in fat tissues.

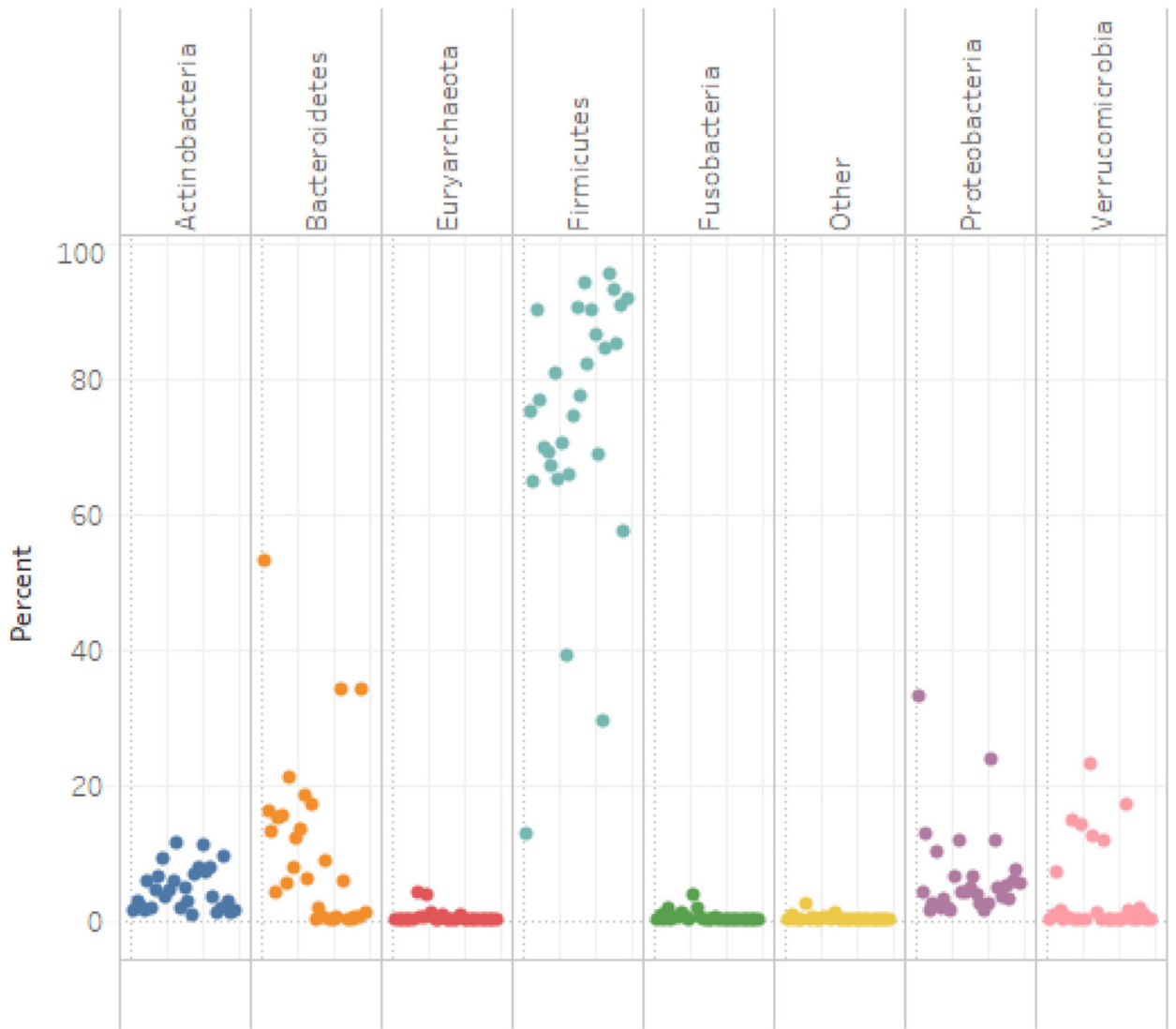


**Figure 2.** Conceptual Framework Exhibiting the Relationship between Fiber Intake, Gut Microbiota and Estrogen Metabolism (Adopted from Kwa, *et al.* 2016) (18).

**Figure 3.**

$\beta$ -glucuronidase encoding bacteria (Adapted from Kwa, et al. 2016) (18).

\*Represents unnamed but previously identified bacterial species/OTUs within the genera



**Figure 4.** Phylum Level Relative Abundance of fecal bacteria from 29 treatment-naïve post-menopausal women with stage 0-II breast cancer.

**Table 1.**

Characteristics of post-menopausal treatment-naïve women with stage 0-II breast cancer ( $n = 29$ ).

Variable	Mean (SD)	Range
Age (years)	62.3 (8.5)	51–85
BMI (kg/m <sup>2</sup> )	34.6 (5.8)	25.9–47.8
Weight (kg)	90.1 (16.6)	58.4–124.7
	N	%
Clinical Stage		
In situ	7	24.1
Invasive	22	75.9
Biopsy Grade		
Low	2	6.9
Low-Intermediate	1	3.5
Intermediate	8	27.6
Intermediate-High	9	31
High	9	31
Hormone receptor status		
Estrogen Receptor Positive	26	89.7
Progesterone Receptor Positive	20	69
Comorbidities		
0	1	3.4
1–2	10	34.5
3+	18	62
Race		
African-American	12	41.4
Non-Hispanic White	15	51.7
More than one race	2	6.9
Cardiovascular disease	4	13.8
Diabetes mellitus	9	31
Smoker	2	6.9

**Table 2.** Dietary fiber intake, fecal microbiota, and serum estradiol concentrations ( $N = 29$ ).

	Mean	SD	Range
Total Dietary Fiber (g/day)	14.22	6.94	5.23 – 39.67
Soluble Dietary Fiber (g/day)	4.54	1.79	2.01 – 8.88
Insoluble Dietary Fiber (g/day)	9.67	5.96	2.32 – 33.81
Serum estradiol (pg/ml)*	17.68	15.12	4.3 – 79.5
Serum estrone (pg/ml)*	198.30	114.43	21.8 – 535.1
<i>Bacteroides</i> :s	2.63%	4.77%	0.002 – 22.301%
<i>Bacteroides</i> :s_ <i>ovatus</i>	0.02%	0.03%	0.000 – 0.157%
<i>Bacteroides</i> :s_ <i>uniformis</i>	0.69%	1.11%	0.000 – 4.352%
<i>Clostridium</i> :s	0.47%	0.51%	0.002 – 1.959%
<i>Clostridium</i> :s_ <i>perfringens</i>	0.01%	0.04%	0.000 – 0.198%
<i>Clostridium</i> :s_ <i>citroniae</i>	0.92%	3.95%	0.000 – 21.321%
<i>Clostridium</i> :s_ <i>hathewayi</i>	0.01%	0.03%	0.000 – 0.106%
<i>Faecalibacterium</i> :s_ <i>prausnitzii</i>	9.24%	8.56%	0.011 – 29.509%
<i>Escherichia</i> :s_ <i>coli</i>	1.63%	4.46%	0.002 – 21.331%
<i>g</i> _ <i>Bifidobacterium</i>	1.26%	2.00%	0.004 – 7.285%
<i>g</i> _ <i>Collinsella</i>	1.48%	1.91%	0.000 – 6.333%
<i>g</i> _ <i>Bacteroides</i>	5.79%	8.63%	0.005 – 31.364%
<i>g</i> _ <i>Lactobacillus</i>	0.69%	1.20%	0.031 – 6.360%
<i>g</i> _ <i>Clostridium</i> ( <i>Clostridiaceae</i> family)	0.56%	0.56%	0.002 – 1.959%
<i>g</i> _ <i>Clostridium</i> ( <i>Lachnospiraceae</i> family)	0.94%	3.95%	0.002 – 21.353%
<i>g</i> _ <i>Roseburia</i>	3.61%	4.03%	0.020 – 16.531%
<i>g</i> _ <i>Faecalibacterium</i>	9.32%	8.64%	0.010 – 29.688%
<i>g</i> _ <i>Clostridium</i> ( <i>Erysipelotrichaceae</i> family)	0.17%	0.44%	0.000 – 1.830%
<i>g</i> _ <i>Escherichia</i>	1.63%	4.46%	0.002 – 21.331%

\* Sample size reduced from 29 to 28 from lack of blood sample for one participant.



Associations between dietary fiber intake, gut microbiota, and serum estradiol concentrations among stage 0-II breast cancer patients ( $n = 29$ ).

**Table 3.**

	Total Dietary Fiber (g/day)	Soluble Dietary Fiber (g/day)	Insoluble Dietary Fiber (g/day)
Serum Estradiol (pg/ml) **	-0.21 (p = 0.28)	-0.30 (p = 0.12)	-0.08 (p = 0.70)
Serum Estrone (pg/ml) **	-0.08 (p = 0.70)	-0.17 (p = 0.40)	0.07 (p = 0.74)
Species			
<i>Bifidobacterium</i> :s_	0.07 (p = 0.71)	0.11 (p = 0.56)	0.02 (p = 0.91)
<i>Bacteroides</i> :s_	0.18 (p = 0.35)	0.00 (p = 1.00)	0.28 (p = 0.14)
<i>Bacteroides</i> :s_ <i>ovatus</i>	0.14 (p = 0.46)	-0.01 (p = 0.96)	0.22 (p = 0.26)
<i>Bacteroides</i> :s_ <i>uniformis</i>	0.30 (p = 0.11)	0.12 (p = 0.54)	<b>0.382*</b> (p = 0.04)
<i>Clostridium</i> :s_	0.17 (p = 0.39)	0.10 (p = 0.62)	0.22 (p = 0.26)
<i>Clostridium</i> :s_ <i>perfringens</i>	0.08 (p = 0.69)	0.06 (p = 0.77)	0.04 (p = 0.83)
<i>Clostridium</i> :s_ <i>citroniae</i>	-0.12 (p = 0.53)	-0.24 (p = 0.22)	-0.08 (p = 0.68)
<i>Clostridium</i> :s_ <i>hathewayi</i>	<b>-0.419*</b> (p = 0.02)	-0.31 (p = 0.10)	-0.35 (p = 0.07)
<i>Faecalibacterium</i> :s_ <i>prausnitzii</i>	0.09 (p = 0.63)	0.00 (p = 0.98)	0.12 (p = 0.54)
<i>Escherichia</i> :s_ <i>coli</i>	0.35 (p = 0.06)	0.14 (p = 0.48)	0.30 (p = 0.12)
Genera			
<i>g__Bifidobacterium</i>	0.27 (p = 0.16)	0.29 (p = 0.13)	0.19 (p = 0.31)
<i>g__Collinsella</i>	-0.08 (p = 0.67)	-0.16 (p = 0.41)	-0.07 (p = 0.70)
<i>g__Bacteroides</i>	0.25 (p = 0.20)	0.06 (p = 0.77)	0.34 (p = 0.07)
<i>g__Lactobacillus</i>	-0.07 (p = 0.71)	-0.12 (p = 0.54)	-0.03 (p = 0.89)
<i>g__Clostridium</i> (Clostridiaceae family)	0.20 (p = 0.29)	0.15 (p = 0.43)	0.23 (p = 0.23)
<i>g__Clostridium</i> (Lachnospiraceae family)	-0.21 (p = 0.28)	-0.22 (p = 0.25)	-0.17 (p = 0.36)
<i>g__Roseburia</i>	-0.08 (p = 0.66)	-0.17 (p = 0.37)	-0.10 (p = 0.62)
<i>g__Faecalibacterium</i>	0.09 (p = 0.63)	0.00 (p = 0.98)	0.12 (p = 0.54)
<i>g__Clostridium</i> (Erysipelotrichaceae family)	-0.16 (p = 0.40)	<b>-0.11*</b> (p = 0.02)	-0.15 (p = 0.44)
<i>g__Escherichia</i>	0.35 (p = 0.06)	0.14 (p = 0.48)	0.30 (p = 0.12)
SUM_Species	0.29 (p = 0.12)	0.22 (p = 0.26)	0.29 (p = 0.13)
SUM_Genera	0.33 (p = 0.08)	0.22 (p = 0.26)	0.31 (p = 0.10)

\* significant at the  $p < .05$  level; these associations were stable after controlling for BMI and age.

<sup>\*\*</sup>  $n = 28$  for this analysis since serum was unavailable for one participant.

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