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RESEARCH ARTICLE

A SYSTEMATIC REVIEW ON RANDOMIZED CONTROL TRIAL(RCT) OF MICROBIAL ALTERATION AND DIET ON BREAST CANCER

Sofia Fathima A [1], Sindhu R[2]*, Haripriya R3, Dinesh Dhamodhar[4], Lubna Fathima[5], Prabu D[6], Rajmohan M [7], Banu Jothi A[8]

[1]Undergraduate, SRM Dental college, Ramapuram, Bharathi salai, Chennai, TN, India.
[2,5] MDS, Senior lecturer, Department of Public health dentistry, SRM Dental college, Ramapuram, Bharathi salai, Chennai, TN, India.
[3,8] Postgraduate, Department of Public health dentistry, SRM Dental college, Ramapuram, Bharathi salai, Chennai, TN, India.
[4,8] MDS, Reader, Department of Public health dentistry, SRM Dental college, Ramapuram, Bharathi salai, Chennai, TN, India.
[7]PhD, Head of the Department, Department of Public health dentistry, SRM Dental college, Ramapuram, Bharathi salai, Chennai, TN, India.

*Corresponding Author
Sindhu R

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Abstract: ***Background***: Breast cancer is a leading malignancy worldwide; emerging evidence links diet and the gut–breast microbiome axis to carcinogenesis and treatment response. ***Methods:*** Using PRISMA, we searched PubMed, EMBASE, CENTRAL, Web of Science, and Wiley from the year 2015–2025 for randomized controlled trials evaluating dietary and microbiota-targeted interventions in breast cancer. Quality was appraised with the Jadad scale. ***Results:*** Three RCTs show that Western-style/high-fat–high-sugar diets drive dysbiosis, endotoxemia, and higher tumour burden. Flaxseed/omega-3–rich strategies attenuated these effects, while safflower oil correlated with more tumour tissue. In women on neoadjuvant chemotherapy, a home-based diet-and-exercise program improved diet quality/physical activity and increased pathologic complete response without harming treatment completion. Fish-oil supplementation reduced Lipopolysaccharides and restored beneficial taxa; broad antibiotics were non-selective. ***Conclusion:*** This study concludes by saying flaxseed oil along with lard diets (FO) are the best experimental high-fat and modified diets, but the plain control diet is also the safest . Modulating diet and the microbiome is a plausible adjunct for breast-cancer prevention and management. Diets emphasizing fibre and omega-3–rich fats may dampen inflammation and tumour-promoting signalling, but evidence remains limited; larger, blinded, longer-duration human Randomised Control Trial with standardized microbiome/metabolomic endpoints are needed for clinical adoption.

Keywords: Breast cancer; Gastrointestinal Microbiome; Diet; Fatty Acids, Dysbiosis

INTRODUCTION

The complex illness known as cancer is typified by unchecked cell division and growth, which results in the development of tumors and their spread (1). Cancer is among the main causes of death worldwide (2). As a result of uncontrolled cell proliferation, cancer develops. Cancers emerge from any organ or body structure and are formed of small cells that have lost the ability to stop growing. Sometimes, a regular radiological examination or laboratory test may "incidentally" detect cancer, or for some other reason(3).

The most prevalent cancer in the world is currently breast cancer, which continues to be a global health concern(4). Breast cancer is the second most important cancer after skin cancer. In 2020, Breast Cancer represented almost 12% of all newly diagnosed cancer cases among 7.8 million women globally(5). As of 2016, India had 118000 incident cases (with a 95% confidence interval between 107000 and 130000), of which 98.1% were female, and 526000 prevalent cases (474,000 to 574000)(6). The most common cancer-related death for women is breast cancer, which accounts for nearly 25% of cancer-related deaths(7). Most breast cancers begin in cells that line the ducts, involved in transporting breast milk to the nipple.e.This specific subtype of breast cancer is referred to as ductal carcinoma. Similarly, cancer can originate from the cells of the lobules, which are groups of glands that produce milk, known as lobular carcinoma(8). Currently, the expression of human epidermal growth factor 2 (HER2), the proliferative index Ki-67, and the HRs estrogen receptor (ER) and progesterone receptor (PR) are used to classify breast cancer into four subgroups clinicopathologically(9).

Due to these alarming figures, it is necessary that strict screening guidelines, preventive and therapeutic measures, and additional research be carried out to better identify the various risk factors for developing breast cancer(10).Breast cancer is caused by a various factors such as a previous breast cancer diagnosis, a family history of the

disease, being overweight, having a taller height, tobacco use, alcohol intake, early onset of menstruation, late onset of menopause, a lack of physical activity, not having children, and the use of hormone replacement therapy(11).Overweight and obesity in women are less probabke to adhere to screening ,guidelines such as mammograms and possess inferior health-related habits(12).Physical activity has many benefits that enhance patient care and reduce risk for disease, so it is important to pre vent and manage BC.Frequent exercise increases resilience and general fitness, which helps patients better withstand treatments like radiation and chemotherapy. This increases the effectiveness of anticancer therapies. (13).Approximately 35% of risk variables are related to diet. Cancer initiation and progression are influenced by diet, which is widely recognized as a moderator of cancer risk(14). According to epidemiologic data, the risk of breast cancer declines when the diet's percentage of whole plant foods rises at the expense of animal and non-whole plant foods.

The Ketogenic Diet is a low-carb, high-fat diet that causes the body's metabolism to change from being based on glucose to ketone metabolism. Numerous preclinical investigations have demonstrated that KD can decrease tumor development, improve BC cells' sensitivity to chemotherapy, and lessen symptoms associated with malignancy(15).A poor, high-fat diet is one of the risk factors for breast cancer. The synthesis and activity of growth factors that support the development of cancer cells are likely inhibited by the Mediterranean diet, which is high in antioxidants and contains trace levels of saturated animal fats(16).Whole plant foods lower the risk of breast cancer by reducing insulin resistance, inflammation, cholesterol, and the bioavailability of sex hormones and insulin-like growth factor 1 compared to animal and non-whole plant foods. They also increase the excretion of fecal estrogen, induce positive changes in the composition and metabolites of the gut microbiota, and may increase bile salt metabolites in the mammary gland and prevent early menarche. (17).Breast cancer is classically managed with surgery, chemotherapy, radiation (RT), endocrine therapy, targeted therapy, and immunotherapy. The treatment regimens require the integration of multiple subspecialties(18).Greater educational initiatives, routine mammography screening programs, and advances in detection methods and treatment protocols make it feasible to detect asymptomatic disease, substantially improving overall survival(19).The incidence and mortality rates have risen over the past thirty years because of shifts in risk factor profiles, improved cancer registration, and advancements in cancer detection.

The human body harbors far more microbial cells (100 trillion) and viruses (quadrillions) than human cells.These microbes constitute our microbiota; the genes they encode are known as the microbiome (20). Microbes have been highlighted for their importance in developing and activating cancer pathology, and there is more evidence linking BC to disturbed microbial balance(21). Breast cancer involves a complicated dynamic connection between the breast microbiome, the gut microbiome, which contains estrogen-metabolizing gut bacteria (the 'estrabolome'), and the immune system(20). The microbiota plays a crucial role in controlling the body's levels of steroid hormones, particularly estrogen. Our knowledge of how gut microorganisms regulate estrogen metabolism has been known for over ten years. However, it is still restricted because of the complexity of the microbiome, which can differ depending on factors including age, race, ethnicity, BMI, and food(22). An imbalance in the microbial community is known as dysbiosis, and it has been linked to several illnesses, including cancer, inflammatory bowel disease, and metabolic syndrome. Research indicates that metabolite synthesis, inflammation, immunological dysregulation, and other mechanisms may be ways microbial dysbiosis contributes to carcinogenesis(23).Breast cancer patients had significantly higher Enterobacteriaceae, Staphylococcus, and Bacillus levels than healthy persons.Additionally, a toxin from the gut-colonizing bacterium Bacteroides fragilis, which is also present in the mammary gland, might cause epithelial hyperplasia to promote the development of tumors(24).Tepidiphilus, Alkanindiges, and Stenotrophomonas were found in samples of invasive ductal carcinoma (IDC), whereas Peptostreptococcus, Micromonospora, Faecalibacterium, and Stenotrophomonas were found in samples of invasive lobular carcinomas (ILC)(25). This study focuses on the microbiome alteration and diet in preventing breast cancer.

MATERIAL AND METHODS

A comprehensive search was performed in the following databases PubMed, EMBASE, Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, Wiley online library to find the related articles using various keyword combinations "Microbiome alteration AND Diet AND Breast Cancer AND RCT", "Microbiome AND Breast Cancer AND RCT", "Diet AND Breast cancer RCT" ("breast cancer" OR "breast neoplasm") AND ("microbiota" OR "probiotics" OR "prebiotics" OR "diet" OR "nutrition") AND ("randomized controlled trial" OR "RCT"). The studies are sorted into years of publishing, and the most relevant articles are chosen. This systematic

review was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines **[Figure 1]**. The risk of bias for each RCT was assessed using the Jadad scale. (25)

Inclusion Criteria:

1. Randomized controlled trials conducted in patients and Animals.
2. Studies focusing on the role of dietary interventions and microbiota modulation.
3. Trials published between 2015 and 2025.

Exclusion Criteria:

1. Observational studies, meta-analyses, or systematic reviews
2. Trials with incomplete or unavailable data

RESULTS AND OBSERVATIONS:

Figure 1: PRISMA 2020 flow diagram for newly conducted systematic reviews that solely involved database and registration searches

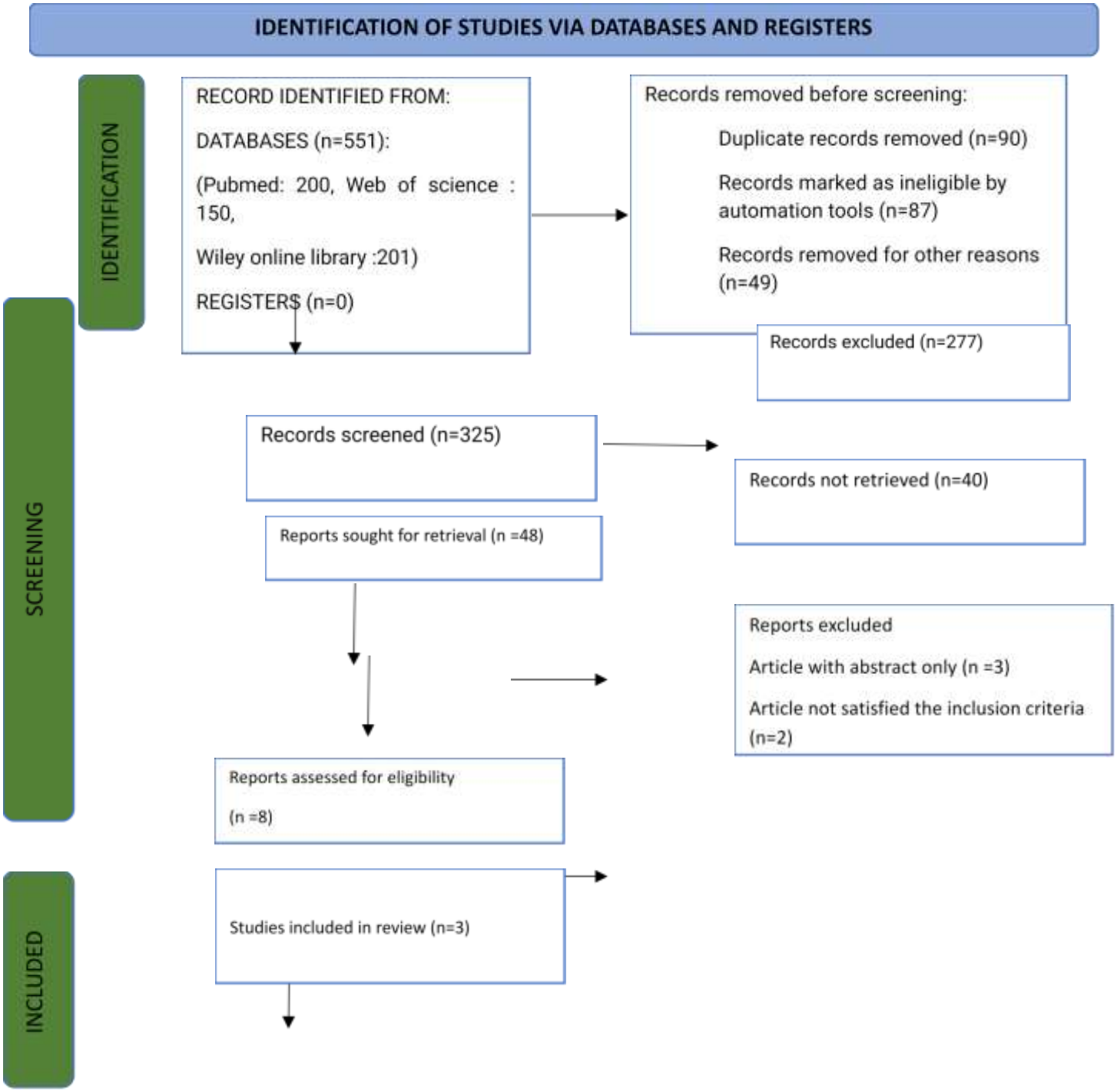

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TABLE 1: Characteristics of Randomized Controlled Trials Evaluating Dietary and Microbial Interventions in Breast Cancer:

Author name	Study design	Sample size	Population	Intervention	Duration/Comparison
Alana A Arnone et.al (26)	Randomized control trial	90	3 Week year balb mice	6 dietary groups (control,high-sugar,lard,coconut oil,lard+flaxseed oil,and lard+safflower oil	Ten weeks
TaraSanft MD et.al (27)	Randomized Control Trial	173	Women	Home based diet and exercise	4 months
David R Soto-pantoja et.al (28)	Randomized control trial	Mice-180 Humans- 22	Mice and women	High fat diet,fecal transplant,antibiotics,fish oil supplements	14.5 days,19 weeks

TABLE 2: Summary of Outcomes from RCTs Investigating the Effects of Microbial and Dietary Alterations on Breast Cancer
Markers and Microbiome Composition

Author	Markers found	Diet involved	Microbiome involved	Result	
				Diet Outcome	Microbiome Alteration
Alana A Arnone et.al (26)	16 sRNA, mammary gland markers, cytometry, Metabolic development, tumor development	High-sugar	Bacteroidetes, Bacteroides, Parabacteroides, Akkermansia, Lactobacillus.	Decreased tumor multiplicity	↑Bacteroidetes (↑Bacteroides, Parabacteroides) with ↓Akkermansia/Lactobacillus
		Lard diet	Firmicutes (Lachnospiraceae, Ruminococcus), Bilophila, Lactococcus, Akkermansia; Lactobacillus.		Akkermansia and Lactobacillus decrease with the lard diet. Also, the Bacteroidetes:Firmicutes ratio decreases
		Coconut-oil	Bilophila, Lactococcus; Lactobacillus;		↑ Bilophila/Lactococcus and ↓ Lactobacillus with Akkermansia

			Akkermansia		being stable.
		Flaxseed-oil + lard	Firmicutes, Akkermansia		↓ Firmicutes & ↓ Akkermansia
		Safflower-oil + lard	Firmicutes; Akkermansia		↓ Firmicutes with Akkermansia maintained
Tara Sanft MD et.al(27)	NIS, RDI, PCR	Plant-based diet	Microbiome not directly involved	No RDI difference; Higher in pcr intervention ; Improved diet and activity; Lower nutritional impact symptoms	No microbiome alteration
David R. Soto-Pantoja et.al (28)	RT-PCR, LPS-endotoxin ELISA, Immunohistochemistry,16s sequencing, Intestinal permeability assay	Lard-based high-fat diet	i)Lactobacillus salivarius; ii)Bacteroides iii)Ruminococcus iv)Alistipes v)Pseudomonas vi)Bacteroides fragilis	On a lard high-fat diet, tumor-free survival drops to 10-12%, with more and heavier tumors that appear sooner. On a control diet receiving fecal microbiota from lard-fed mice, tumor-free survival likewise falls to ~10–12% and tumor counts rise, mimicking the lard diet. On a lard diet receiving control-mouse tumor-free survival improves to ~50% and tumor weight is lower than lard-only. In mice on a Western diet plus fish oil, tumor numbers weren't reported, but fish oil reduced systemic and mammary LPS and improved barrier/polarity markers.	The lard diet (or receiving lard-FMT) shifted microbes (↓Bacteroidetes, ↑Firmicutes; ↑Akkermansia/Butyricimonas/Lachnospiraceae signatures, ↓ Verrucomicrobia, ↓ Lactobacillus, ↑Ruminococcus, Bilophila, Lactococcus, ↑ Proteobacteria.) and tumors showed more gram-positive and LPS-positive bacteria

Note: NIS — Nutrition Impact Symptoms (patient-reported symptom score);**RDI** — Relative Dose Intensity (of chemotherapy); **PCR** — *Pathologic* Complete Response; **RT-PCR** — Reverse Transcription Polymerase Chain Reaction; **LPS** — Lipopolysaccharide; **ELISA** — Enzyme-Linked Immunosorbent Assay

TABLE3: Bias assessment by Jadad Scale Assessment for the Studies:

Article	Randomized	Appropriate Randomization	Blinded	Appropriate Blinding	Withdrawals Described	Total Score (out of 5)
Arnone et al. (2024)	Yes	Yes	No	No	Yes	3
Sanft et al. (2023)	Yes	Yes	No	No	Yes	3
Soto-Pantoja et al. (2021)	Yes	Yes	No	No	Yes	3

Note: Yes = Criteria met, No = Criteria not met. The Jadad scale ranges from 0–5, where higher scores indicate lower risk of bias and better methodological quality. This research resulted in 48 articles, of which 8 were full-text articles having accessibility and were eligible for review. Ultimately, 3 articles were chosen for inclusion in this systematic review. Table **1** shows the Characteristics of Randomized Controlled Trials Evaluating Dietary and Microbial Interventions in Breast Cancer. **Table 2** shows summary of Outcomes from RCTs Investigating the Effects of Microbial and Dietary Alterations on Breast Cancer Markers and Microbiome Composition. The **table 3** represents the quality assessment of three included studies using the Jadad scale, which evaluates the risk of bias across five key domains: randomization, randomization appropriateness, blinding, blinding appropriateness, and withdrawals. All three studies demonstrated a robust approach to randomization and appropriately conducted this process, yielding a "Yes" for both relevant criteria. This suggests that subject assignment was conducted employing methodologically sound methods in the studies. No study used or reported any blinding practices, nor did they detail blinding procedures. This deficiency increases the possibility of performance and detection bias since both the participants and researchers were aware of the interventions and outcomes assigned to them. However, the three studies all reported participant withdrawals and dropouts in a transparent manner in terms of managing attrition. Each Study received an overall Jadad score of three out of five, indicating moderate quality. The results show strengths in randomization and follow-up of participants, but also identify blinding as an area for important future improvement. Generally, though these studies are very informative and reflect good practice in randomization and dealing with attrition, the absence of blinding is a limitation that one must consider while assessing the findings.

DISCUSSION

This systematic review states that diet is crucial to breast cancer risk by modifying the gut and breast microbiomes through entero-mammary communication. High-fat, high-sugar diets increase harmful bacteria and circulating endotoxins like lipopolysaccharides (LPS), which damage breast tissue barriers, promote inflammation, and support tumor growth. Animal studies show that gut microbes from high-fat diet mice accelerate breast cancer, whereas antibiotics reduce tumors. In humans, supplements such as fish oil can alter the breast microbiome, suggesting diet-based strategies may help prevent or slow cancer progression. Conversely, healthy diets like the Mediterranean diet encourage beneficial microbes, boost anticancer bile acids, lower local estrogen activity, and reduce inflammation in breast tissue, collectively reducing cancer risk. Clinical research, such as the LEANer Study, indicates that lifestyle interventions combining nutrition and exercise during chemotherapy can improve tumor response rates, particularly in neoadjuvant settings, while enhancing diet quality and physical activity.

According to Arnone et al.(2024), diet influences the risk of breast cancer by altering metabolism, inflammation of the mammary glands, and gut microbiota. Sugar and unhealthy fat-rich diets can change the gut microbiota and raise the risk of breast cancer. By altering the microbiome, a Mediterranean diet may have anti-breast cancer effects. A Western diet heavy in animal fats may increase the risk of breast cancer. More research is required to understand dietary-microbiome interactions and how they relate to the prevention and treatment of breast cancer.

In the LEANer Study of Tara Sanft et al (2023) found that home-based exercise and nutrition interventions during chemotherapy failed to increase completion rates of chemotherapy. However, the intervention significantly enhanced the pathologic complete response rate (53% vs. 28%) among women undergoing neoadjuvant chemotherapy. Additionally, the program produced notable improvements in nutrition and physical activity, indicating that lifestyle alterations may increase the tumors' response to treatment.

David R Soto Pantoja et al(2021) have described in their Study that diet has a significant impact on the gut and breast microbiomes, which in turn affects the risk and prognosis of breast cancer. Studies reveal that eating habits can alter the populations of mammary microbiota, which can affect the development of tumors. A diet heavy in fat can cause protumorigenic effects; however, dietary supplements containing fish oil can change the microbiota in breast tissue and tumors. Gaining insight into the connection between gut microbiota, food, and breast cancer risk could result in the development of innovative dietary therapies for both prevention and treatment.

These studies point to a bidirectional communication system between the gut and breast tissue, where microbial signals can influence tumor biology. High-fat and high-sugar diets create a dysbiotic environment that favors inflammation and carcinogenesis, while dietary fiber, healthy fats, and exercise promote microbial diversity and anti-inflammatory effects. Additionally, evidence from the supporting literature (e.g., Samami et al. and Kudiarasu et al.) (29,30) and its data support the importance of dietary fiber and organized exercise in preserving good body composition, hormonal equilibrium, and microbial balance. These interventions have been promising concerning survival enhancement and recurrence diminishment, along with favorable treatment results among breast cancer patients. A lard diet is a lab high-fat diet where pork fat (lard) is the main lipid source, typically ~60% of calories from fat.

Lard is rich in oleic (mono-unsaturated) plus palmitic/stearic (saturated) fatty acids, so it's used to model "Western-style" fat exposure(31). The flaxseed oil along with lard diet (FO) is the best among the experimental high-fat / modified diets, but the plain control diet is still the safest overall. The plant-forward, high-fiber, low–added-sugar/red-meat approach is best—because it's the only one with human RCT evidence of better tumor response.The limitations of the studies are small sample sizes, heterogeneity of dietary interventions, limited durations of follow-up, and restricted direct microbial sequencing in humans. The mechanistic data were based on animal models, but would need to be translated and verified in human environments.

Conclusion:

This review highlights the important role of interactions between diet and the microbiome in preventing and developing breast cancer. This study concludes by saying flaxseed oil along with lard diets (FO) are the best experimental high-fat and modified diets, but the plain control diet is also the safest. When integrated with lifestyle interventions, personalized nutrition strategies targeting microbial modulation could form the basis for innovative preventive and therapeutic approaches in oncology. Future clinical trials with larger cohorts, extended follow-ups and integrated microbiome profiling are warranted to develop precision nutrition and microbiota-based therapies in breast cancer care.

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