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Randomized Controlled Trial

An empirically derived dietary pattern associated with breast cancer risk is validated in a nested case-control cohort from a randomized primary prevention trial

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SUMMARY

Background & aims: We reported an association between cytologic atypia, a reversible biomarker of breast cancer risk, and lower omega-3/omega-6 fatty acid ratio in blood and breast tissue. Our goal was to develop and validate a dietary pattern index in this high-risk sample of U.S. women, and test its capacity to predict incidence in a nested case-control cohort of Canadian women from a randomized trial of a low-fat dietary intervention for primary prevention of breast cancer.

Methods: Food intake was measured by food frequency questionnaire in the U.S. sample (n = 65) and multiple dietary recalls in the Canadian sample (n = 220 cases; 440 controls). Principal component analysis identified a dietary pattern associated with atypia. We measured differences among dietary pattern tertiles in (a) fatty acid composition in blood lipids and breast tissue in the U.S. sample, and (b) risk of breast cancer subtypes in the Canadian cohort. Registered under ClinicalTrials.gov Identifier: NCT00148057.

Results: A Modern diet was characterized as consuming more grains, dairy, and sugar and less vegetables, fish and poultry; these women had lower tissue omega-3 fatty acids and higher omega-6 and *trans* fatty acids. The low-fat intervention increased the likelihood of a Modern diet after randomization. A Modern diet at baseline and post-randomization was associated with estrogen-receptor negative (ER−) breast cancer risk among those at least 160 cm tall. A Traditional diet (the reciprocal of Modern) at baseline was associated with lower ER-positive (ER+) risk in the comparison group, but not the low-fat intervention group.

Conclusions: A Modern diet (high in grains, dairy, and sugar and low in vegetables, fish, and poultry) is associated with ER− breast cancer risk among taller women. Recommending dietary fat reduction may have untoward effects on breast cancer risk.

1. Introduction

Diet is a long considered modifiable risk factor for mammary tumor development [1], however, the first evidence of a dietary intervention reducing breast cancer incidence came from a recently reported randomized controlled trial among women at high cardiovascular risk [2]. Women randomly assigned to follow a Mediterranean dietary pattern high in fish and plant foods (especially if supplemented with extra virgin olive oil) were about half as likely to develop invasive breast cancer as those who only received advice to reduce dietary fat intake [2].

Abbreviations: DHA, docosahexaenoic acid; DHQ-1, diet history questionnaire-I; EPA, eicosapentaenoic acid; ER, estrogen receptor; LCPUFA, long chain polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; NDS-R, nutritional data system for research; PCA, principal components analysis; OR and aOR, odds ratio and adjusted odds ratio; PL, phospholipids; PUFA, polyunsaturated fatty acids; RPFNA, random periareolar fine needle aspiration; TAG, triacylglycerides.

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In fact, the two largest trials to study the effects of a low-fat diet on primary prevention did not find that a low-fat diet reduced risk overall [3,4]; however, there was evidence that the intervention lent protection against developing an estrogen-receptor positive (ER+) tumor.

The aforementioned randomized trials were designed and implemented before fat quality, i.e., the type of fatty acids, was understood to be an important determinant of breast cancer risk effects. Moreover, burgeoning evidence suggests breast cancer subtypes have different risk factor profiles [5]. For example, saturated fatty acid intake is associated with a higher risk of ER+ breast cancer [6], while monounsaturated fatty acids (MUFA) intake is not [6]. In fact, olive oil is an important source of MUFA, and greater consumption of olive oil is associated with lower overall breast cancer risk [2]. Higher intake of long chain n-3 polyunsaturated fatty acids (LCPUFA), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is also associated with lower overall breast cancer risk [7] and we associated breast atypia with lower blood and breast n-3 fatty acids [8]. Higher tissue levels of *trans* fatty acids, mostly obtained from foods that contain partially hydrogenated oils, are also associated with elevated breast cancer risk [9].

A focus on a single nutrient such as fat reflects in part the difficulty of condensing overall dietary intake. Recently, multivariate statistics have become increasingly popular tools to study how dietary behavior relates to breast cancer risk [10], because they generate dietary patterns that incorporate the complex interactions among foods and nutrients. However, this methodology is beset by two major barriers: (a) ambiguous interpretation of dietary pattern meaning and (b) difficulty generalizing to other populations.

This study addresses both of these shortcomings by empirically deriving a dietary pattern, and reifying it with biomarkers of intake (tissue fatty acids) in a smaller cohort of women at increased risk of developing breast cancer; and subsequently testing the dietary pattern's capacity to predict risk of breast cancer subtypes in a separate sample of similarly aged women.

2. Materials and methods

2.1. Midwestern U.S. sample eligibility and data collection

Premenopausal and postmenopausal women at an elevated risk of developing breast cancer were eligible for random periareolar fine needle aspiration (RPFNA) at the Breast Cancer Prevention Center research clinic at the University of Kansas Medical Center. Women were deemed at elevated risk based on meeting at least one of the following criteria: (a) one first-degree relative, or two second-degree relatives, that were diagnosed with breast cancer before age 60, (b) a prior breast biopsy diagnosed as atypical hyperplasia or ductal/lobular carcinoma *in situ*, (c) multiple biopsies from suspicious mammograms, or (d) $\geq$50% mammographic breast density. Participants signed an informed consent document approved by the University of Kansas Medical Center Human Subjects Committee, which is responsible for institutional ethical standards.

Information for estimating individual breast cancer risk using the Gail model [11] was collected from participants by phone prior to visiting the clinic. The Gail risk model is calculated from current age, age of menarche, age at the time of the birth of the first child (or nulliparous), family history of breast cancer, number of breast biopsies, number of breast biopsies showing atypical hyperplasia, race, and ethnicity. Height was measured by a stadiometer to the nearest millimeter. Weight was measured by an electronic scale to the nearest 100 g.

Participants completed the National Cancer Institute's (NCI) food frequency questionnaire, the Diet History Questionnaire-I (DHQ-I) [12]. Responses to the multiple-choice questions were entered into Diet*Calc version 1.4, a software program developed by the NCI to analyze the DHQ-I. Input was computed into average daily nutrient intake using calculations from the Nutritional Data System for Research (NDS-R) and United States Department of Agriculture National Nutrient Database for Standard Reference, release 22. The output of most dietary parameters (123 of 131) was as weight or servings per day. Other variables included glycemic load, total energy intake, the percent energy contribution of carbohydrates, fat (with subtypes), and protein.

RPFNA is a method for sampling benign breast tissue to detect a validated risk biomarker, cytologic atypia, which confers five times the risk of developing carcinoma *in situ* or invasive breast cancer over the next four years [13]. Details of tissue harvest by RPFNA, storage, processing, and fatty acid analysis are described elsewhere [8]. Briefly, a cytopathologist rated specimens stained with hematoxylin and eosin as nonproliferative, hyperplasia without atypia, hyperplasia with atypia, or possible malignancy.

Venous blood (not fasting) was kept on ice or refrigerated at 4 °C as it was separated into plasma and erythrocytes and stored in cryovials at −80 °C until thawed for fatty acid analysis. Thin layer chromatography separated phospholipids (PL) from triacylglycerides. Plasma phospholipids (PL) and triacylglycerides (TAG) were isolated from plasma, PL from red blood cells (RBC), and TAG from breast adipose via RPFNA. The fatty acid composition of various lipid compartments of blood and adipose tissue serve as a lens into the types of dietary fat consumed over different time frames. Plasma TAG indicates intake over hours to days, plasma PL days to weeks, RBC PL weeks to months, and breast TAG months to years [14]. After lipid isolation and transmethylation, fatty acid methyl ester products were analyzed by gas chromatography as reported previously [15]. Forty fatty acids were measured in each lipid compartment as percent of total fatty acids.

2.2. Canadian nested case-control sample

Complete information about this subset of participants from a randomized controlled trial investigating a low-fat dietary intervention for primary prevention of breast cancer is available in the trial's primary results [3]. The trial was registered under ClinicalTrials.gov Identifier: NCT00148057. Women were eligible to participate in the trial if they were aged 30 to 65 y, had >50% mammographic density, had no personal history of cancer, had a body mass index (BMI) between 19 and 27, and were not pregnant or breast-feeding. Women were recruited from eight sites in Canada. Participants were allocated in a one-to-one ratio. Participants randomized to the active intervention group received one-on-one counseling to replace dietary fat with carbohydrates toward a goal of 15% of energy coming from fat; these women were seen monthly for the first year, quarterly for the second, and biannually thereafter. The comparison group was given general governmental dietary advice. They were seen quarterly for the first year, twice in the second year, and annually thereafter. Women were followed for an average of 10 y, with a minimum of 7 y.

Fig. 1 shows a flow chart of participants.

About 16% of those invited to participate expressed interest; of those who expressed a willingness to be screened, 40% underwent randomization. After randomization, 12% of participants withdrew from the trial, which was more common in the low-fat group: OR 1.40 (95% CI: 1.17 to 1.67). The nested case-control sample for this analysis is comprised of the 220 incident invasive breast cancer (BC) cases individually matched to two controls by age, date of randomization, center of randomization, and duration of follow-up.

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