

Insulinemic and Inflammatory Dietary Patterns and Breast Cancer Outcomes: Role of the PI3K/AKT/MTOR Pathway Among Black Women

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Abstract

Word Limit 243/250 Overactivation of the PI3K/AKT/MTOR pathway may contribute to breast cancer (BrCa) progression and mortality, but whether dietary patterns influence this pathway is unclear. Immunohistochemical markers (MTOR, phosphorylated [p]-MTOR, p-AKT, p-P70S6K) were assessed in surgical tissue from 394 Black women in the Women's Circle of Health Study (WCHS) and WCHS Follow-up Study (WCHFS). Dietary scores for the reversed Empirical Dietary Index for Hyperinsulinemia (rEDIH), reversed Empirical Dietary Inflammatory Pattern (rEDIP), and Alternative Healthy Eating Index (AHEI-2010) were calculated from food frequency questionnaires. Multivariable logistic regression estimated odds ratios (ORs) for associations with marker positivity, and Cox regression evaluated dietary pattern–mortality associations. Each 1-standard deviation (SD) increment in rEDIH was associated with 24% lower odds of p-P70S6K-positive tumors (OR=0.76; 95% CI: 0.58, 0.98; P=0.032). Among women with p-MTOR-positive cancers, higher rEDIH was associated with lower all-cause (HR=0.82; 95% CI: 0.71, 0.95; P=0.008) and BrCa-specific mortality (HR=0.80; 95% CI: 0.69, 0.92; P=0.002). Similar associations were observed among women with p-AKT-and p-P70S6K-positive cancers. Higher rEDIP was associated with lower BrCa-specific mortality among women with p-AKT-and p-P70S6K-positive cancers but was not associated with marker positivity. AHEI-2010 was not associated with marker status or mortality. A low-insulinemic dietary pattern, reflected by higher rEDIH, was associated with lower odds of p-P70S6K positivity and lower mortality among Black women with tumors expressing PI3K/AKT/MTOR pathway markers. These findings suggest mechanistically targeted dietary indices may better capture pathways relevant to BrCa progression than overall diet quality.

INTRODUCTION

Breast cancer (BrCa) is the most commonly diagnosed cancer among women in the United States,¹ and while mortality rates have declined steadily over the past decade,^{2,3} incidence continues to rise.⁴ Despite improvements in 5-year survival rates,⁵ BrCa-specific mortality among Black women remains approximately 1.4 times higher than that among White women.³

These disparities are driven in part by differences in modifiable risk factors, including obesity, energy imbalance, and diet.⁶ Obesogenic dietary patterns⁷ such as those captured by the Empirical Dietary Index for Hyperinsulinemia (EDIH)⁸ and the Empirical Dietary Inflammatory Pattern (EDIP)⁹ are associated with increased risk of invasive, estrogen receptor-negative, HER2-, and triple-negative BrCa.^{10,11} Notably, hyperinsulinemic diet consumption was 3.5 times more prevalent among Black women in the highest versus lowest EDIH quintile, underscoring marked racial differences in dietary exposure to chronically high levels of insulin.¹⁰ Higher dietary quality, such as those assessed by the Alternative Healthy Eating Index (AHEI), are associated with a decreased risk of incident breast cancer among postmenopausal women¹² and a reduced risk of breast cancer mortality.¹³

Energy imbalance is also implicated in overactivation of the PI3K/AKT/MTOR pathway.^{14–17} Under normal conditions, this pathway regulates protein synthesis, cellular metabolism, immune response, glucose uptake, and apoptosis.^{18,19} When aberrantly activated or when the tumor suppressor PTEN is lost,^{20,21} it drives uncontrolled proliferation, differentiation, suppression of apoptosis, and angiogenesis.^{19,22,23}

Although EDIH, EDIP, and the PI3K/AKT/MTOR pathway have each been independently linked to BrCa risk and prognosis, their interactions have not been examined. We hypothesized that low-insulinemic, anti-inflammatory, and higher-quality dietary patterns are associated with reduced BrCa-specific and all-cause mortality, mediated through attenuation of PI3K/AKT/MTOR pathway overactivation.

METHODS

Study Participants

The Women's Circle of Health Study (WCHS) was a multi-site case-control study enrolling women from New York City and 10 New Jersey counties between 2003 and 2013.²⁴ Cases were self-identified Black or White women aged 20–75 years with no prior cancer history (except nonmelanoma skin cancer) and a histologically confirmed breast cancer diagnosis. At enrollment, participants completed interviewer-administered questionnaires, including a food frequency questionnaire (FFQ), had anthropometric measurements taken, and consented to release of pathology reports and tumor tissue samples.²⁴ Follow up data were obtained through the WCHS Follow-Up Study (WCHFS), a prospective cohort that continued to recruit Black women in the same 10 counties in New Jersey from 2014 to 2020 using similar methodology and followed all cases for mortality outcomes.^{25,26} The current analysis includes 394 Black women with both a completed baseline FFQ and immunohistochemical (IHC) biomarker data from tumor tissue. Both studies were approved by the Institutional Review Boards at Roswell Park Comprehensive Cancer Center and Rutgers Cancer Institute.^{24,26}

Diet Assessment and score computation

Diet was assessed using validated semi-quantitative FFQs at baseline: the 125-item Fred Hutchinson Cancer Research Center FFQ and, since 2012, the 85-item Black Women's Health Study FFQ with slight modifications; both captured average intake over the 12 months prior to diagnosis with standard portion sizes.^{24,26,27} Insulinemic and inflammatory dietary quality were assessed using the reversed EDIH (rEDIH) and reversed EDIP (rEDIP), respectively; overall dietary quality was assessed using the Alternative Healthy Eating Index (AHEI-2010). Both rEDIH and rEDIP comprise 18 food groups, nine shared between them, including red meat, processed meat, non-oily fish, green leafy vegetables, tomatoes, low- and high-energy beverages, wine, and coffee.^{8,9} Higher rEDIH and rEDIP scores, ranging from – 3.30 to -0.35 and from – 2.07 to 0.31 in a cohort of one million individuals for rEDIH and rEDIP respectively²⁸, indicate lower insulinemic and anti-inflammatory potential; scores were reversed so that higher values align directionally with higher AHEI-2010 scores (range: 0–110), facilitating interpretation.^{29,30}

Immunohistochemical Staining and Analysis

Tumor tissue microarrays (TMAs) were assayed for MTOR, phosphorylated (p)-MTOR, p-AKT, and p-P70S6K as markers of PI3K/AKT/MTOR pathway activation.^{14,15} Formalin-fixed paraffin-embedded (FFPE) tissue from 2–3 tumor cores (0.6–1.2 mm) per participant were used for TMA construction. Sections cut at 5µm were rehydrated, prepared in high-pH solution for 30 minutes, then incubated with primary antibodies: MTOR (7C10), p-MTOR (Ser2448), p-AKT (Ser743), and p-P70S6K (T389), for 40 minutes (MTOR, p-MTOR, p-P70S6K) or 16 hours (p-AKT), followed by secondary polymer application. Slides were digitally imaged at 20x using the Aperio ScanScope XT scanner (Leica Biosystems) and were manually annotated for tumor analysis. Analysis of the annotated regions was conducted via Aperio's algorithm which was verified by using positive and negative controls of tissue sample with differing levels of stain intensity. H-scores were computed as the weighted average of percent positivity across intensity categories (0–3+): $H\text{-Score} = [1 \times (\%1+) + 2 \times (\%2+) + 3 \times (\%3+)] \times 100$.^{14,15} MTOR and p-MTOR were assessed in the cytoplasm; p-AKT and p-P70S6K in both cytoplasm and nucleus.

Ascertainment of Covariates

Baseline covariates included age, total energy intake, waist-hip ratio, education, age at menarche and menopause, oral contraceptive and hormone replacement therapy use, parity, breastfeeding, reproductive years, smoking, alcohol use, benign breast disease history, family history of BrCa and cancer, recreational physical activity,³¹ diabetes history, and BMI.

Statistical Analysis

Dietary scores were energy-adjusted using the residual method³² and categorized into quartiles (lowest = reference). Spearman correlation coefficients assessed the relationship between each dietary score and BMI. IHC markers were dichotomized as positive (H-score > 10) or negative (H-score ≤ 10). Multivariable logistic regression estimated odds ratios for the associations between dietary scores (per quartile and per 1-SD increment) and IHC marker expression. Continuous H-score associations were examined using log-transformed values in linear regression, with results expressed as percentage differences.

Multivariable-adjusted Cox proportional hazards models estimated all-cause and BrCa-specific mortality risk per 1-SD increment in each dietary score, overall and stratified by IHC marker status (positive vs. negative). All models adjusted for the covariates listed above.

Because both FFQs were validated for nutrient intake,^{33,34} captured comparable food groups, and allowed comparability with prior WCHS and WCHFS analyses, dietary scores were derived from the data to prioritize the original validated assessments. In sensitivity analyses, dietary scores were additionally computed using Nutrition Data System for Research (NDSR)³⁵ as a reference to convert grams of food intake to Dietary Guidelines for Americans (DGA) serving sizes to assess robustness to an alternative cross-FFQ harmonization approach and multivariable-adjusted Cox proportional hazards models estimated all-cause and BrCa-specific mortality risk further accounting for tumor characteristics and treatment modalities..²⁸ Analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC) and R Studio (2024.12.0); statistical significance was set at $\alpha = 0.05$.

RESULTS

Participant Characteristics

The mean age at baseline was 52.5 (SD = 11.4) years. Median dietary scores unadjusted for total energy intake, ranged from – 1.10 to -0.17 for rEDIH, -0.68 to 0.29 for rEDIP, and 45.7 to 72.4 for AHEI-2010 across quartiles. Participants in the highest quartile of each score were less likely to be obese or current smokers, and more likely to have a college degree, have used hormone therapy, and engage in moderate or vigorous physical activity. Women in the highest quartiles of rEDIH and rEDIP were also more likely to breastfeed (among parous women), have a first-degree relative with breast cancer or any cancer, have lower-grade tumors, but were less likely to have diabetes; these patterns were less consistent across AHEI-2010 quartiles (Table 1). Spearman correlations between dietary scores were 0.49 (rEDIH-rEDIP), 0.38 (rEDIH-AHEI-2010), and 0.34 (rEDIP-AHEI-2010).

Table 1

Distribution of Participant Characteristics in Quartiles of Dietary Indices^a

Characteristic	Reverse Empirical Dietary Index for Hyperinsulinemia (REDIH) Score ^b					Reverse Empirical Dietary Inflammatory Pattern (REDIP) Score ^b			
	Total (n = 394)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)
Dietary Score, median (range)		-1.11 (10.03)	-0.46 (1.94)	-0.30 (2.74)	-0.17 (3.30)	-0.68 (4.66)	-0.22 (0.79)	0.00 (0.70)	0.29 (4.32)
Age (years), mean (SD)	52.5 (11.4)	49.3 (11.2)	52.1 (12.0)	54.3 (10.6)	54.5 (11.0)	51.2 (11.7)	52.3 (11.4)	53.6 (11.4)	53.0 (10.9)
BMI (kg/m²), mean (SD)	31.8(6.9)	33.1(6.8)	31.3(7.1)	32.4(7.2)	30.3(6.4)	32.1(8.0)	31.8(6.4)	31.3(6.5)	31.8(6.9)
BMI (kg/m²) category, n (%)									
BMI < 25	56 (14.2)	11 (11.2)	16 (16.2)	11 (11.1)	18 (18.4)	15 (15.3)	12 (12.1)	16 (16.2)	13 (13.3)
25 ≤ BMI < 30	121 (30.7)	22 (22.5)	34 (34.3)	33 (33.3)	32 (32.7)	29 (29.6)	30 (30.3)	30 (30.3)	32 (32.7)
BMI ≥ 30	217 (55.1)	65 (66.3)	49 (49.5)	55 (55.6)	48 (48.9)	54 (55.1)	57 (57.6)	53 (53.5)	53 (54.0)
Waist-to-Hip Ratio, mean (SD)	0.88 (0.08)	0.89 (0.06)	0.88 (0.08)	0.89 (0.07)	0.88 (0.08)	0.89 (0.09)	0.88 (0.07)	0.88 (0.07)	0.89 (0.07)
Education, n (%)									
High School or Less	176 (44.7)	45 (45.9)	42 (42.4)	49 (49.5)	40 (40.8)	54 (55.1)	47 (47.5)	41 (41.4)	34 (34.7)
Some College	106 (26.9)	28 (28.6)	21 (21.2)	24 (24.2)	33 (33.7)	18 (18.4)	30 (30.3)	27 (27.3)	31 (31.6)
College Graduate or More	112 (28.4)	25 (25.5)	36 (36.4)	26 (26.3)	25 (25.5)	26 (26.5)	22 (22.2)	31 (31.3)	33 (33.7)
Age at Menarche (years), mean (SD)	12.4(2.0)	12.2(1.5)	12.6(2.5)	12.5(1.9)	12.3(1.9)	12.4(1.8)	12.7(1.8)	12.5(2.4)	12.0(1.8)
Oral Contraceptive Use, n (%)^c									
Yes	275 (70.0)	65 (66.3)	72 (72.7)	72 (72.7)	66 (68.0)	68 (69.4)	62 (62.6)	69 (70.4)	76 (77.6)
No	118 (30.0)	33 (33.7)	27 (27.3)	27 (27.3)	31 (32.0)	30 (30.6)	37 (37.4)	29 (29.6)	22 (22.4)
Number of Full-Term Pregnancies, mean (SD)	2.1 (1.5)	1.9 (1.4)	2.3 (1.5)	2.2 (1.6)	2.1 (1.4)	2.3 (1.4)	2.3 (1.7)	2.0 (1.5)	1.9 (1.3)
Breastfeeding, n (%)									
Nulliparous	53 (13.5)	17 (17.4)	10 (10.1)	11 (11.1)	15 (15.3)	9 (9.2)	11 (11.1)	16 (16.2)	17 (17.4)
Yes	140 (35.5)	34 (34.7)	36 (36.4)	31 (31.3)	39 (39.8)	31 (31.6)	34 (34.3)	35 (35.3)	40 (40.8)
No	201 (51.0)	47 (47.9)	53 (53.5)	57 (57.6)	44 (44.9)	58 (59.2)	54 (54.6)	48 (48.5)	41 (41.8)
Number of Reproductive Years, mean (SD)	33.8 (7.1)	33.5 (7.5)	33.1 (7.7)	34.3 (6.5)	32.3 (6.6)	33.0 (7.2)	33.3 (7.1)	34.2 (6.8)	34.7 (7.2)

Characteristic	Reverse Empirical Dietary Index for Hyperinsulinemia (rEDIH) Score ^b					Reverse Empirical Dietary Inflammatory Pattern (rEDIP) Score ^b			
	Total (n = 394)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)
Age at Menopause (year), mean (SD) ^d	49.1 (5.4)	50.6 (4.4)	49.2 (5.6)	48.7 (5.7)	48.3 (5.4)	49.0 (5.7)	49.1 (5.7)	49.1 (5.2)	49.2 (4.9)
Cause of Menopause, n (%)									
Premenopausal	175 (44.4)	53 (54.1)	45 (45.4)	40 (40.4)	37 (37.8)	47 (48.0)	46 (46.5)	43 (43.4)	39 (39.8)
Naturally Occurring	108 (27.4)	26 (26.5)	27 (27.3)	24 (24.2)	31 (31.6)	23 (23.5)	20 (20.2)	34 (34.4)	31 (31.6)
Other Cause ^e	111 (28.2)	19 (19.4)	27 (27.3)	35 (35.4)	30 (30.6)	28 (28.6)	33 (33.3)	22 (22.2)	28 (28.6)
Hormone Replacement Therapy Use, n (%) ^f									
Yes	56 (14.4)	8 (8.3)	13 (13.3)	12 (12.2)	23 (23.7)	10 (10.2)	15 (15.5)	13 (13.3)	18 (18.6)
No	334 (85.6)	89 (91.7)	85 (86.7)	86 (87.8)	74 (76.3)	88 (89.8)	82 (84.5)	85 (86.7)	79 (81.4)
Smoking Status, n (%)									
Never	233 (59.1)	57 (58.1)	61 (61.6)	45 (45.5)	70 (71.4)	56 (57.1)	56 (56.6)	64 (64.7)	57 (58.2)
Former	100 (25.4)	23 (23.5)	24 (24.2)	33 (33.3)	20 (20.4)	26 (26.5)	22 (22.2)	22 (22.3)	30 (30.6)
Current	61 (15.5)	18 (18.4)	14 (14.2)	21 (21.1)	8 (8.2)	16 (16.4)	21 (21.2)	13 (13.1)	11 (11.2)
At Least One Alcoholic Beverage Per Week, n (%) ^g									
Yes	155 (39.3)	38 (38.8)	37 (37.4)	41 (41.4)	39 (39.8)	37 (37.8)	35 (35.4)	38 (38.4)	45 (45.9)
No	239 (60.7)	60 (61.2)	62 (62.6)	58 (58.6)	59 (60.2)	61 (62.2)	64 (64.6)	61 (61.6)	53 (54.1)
Physical Activity, Moderate Intensity n (%) ^h									
Sufficient	92 (23.4)	19 (19.4)	25 (25.3)	17 (17.2)	31 (31.6)	26 (26.5)	19 (19.2)	19 (19.2)	28 (28.6)
Insufficient	62 (15.7)	19 (19.4)	13 (13.1)	13 (13.1)	17 (17.4)	16 (16.3)	16 (16.1)	14 (14.1)	16 (16.3)
No Moderate Physical Activity	240 (60.9)	60 (61.2)	61 (61.6)	69 (69.7)	50 (51.0)	55 (57.1)	64 (64.7)	66 (66.7)	54 (55.1)
Physical Activity, Vigorous Intensity n (%) ^h									
Sufficient	59 (15.0)	11 (15.3)	11 (11.1)	13 (13.1)	20 (20.4)	9 (9.2)	12 (12.1)	18 (18.2)	20 (20.4)
Insufficient	17	8	4	1	4	7	2	3	5

Characteristic	Reverse Empirical Dietary Index for Hyperinsulinemia (rEDIH) Score ^b					Reverse Empirical Dietary Inflammatory Pattern (rEDIP) Score ^b			
	Total (n = 394)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)
	(4.3)	(8.2)	(4.0)	(1.0)	(4.1)	(7.1)	(2.0)	(3.0)	(5.1)
No Vigorous Physical Activity	318 (80.7)	75 (76.5)	84 (84.9)	85 (85.9)	74 (75.5)	82 (83.7)	85 (85.9)	77 (78.8)	73 (74.5)
1st Degree Relative with Breast Cancer, n (%)									
Yes	65 (16.5)	15 (15.3)	13 (13.1)	17 (17.2)	20 (20.4)	13 (13.3)	12 (12.1)	19 (17.2)	21 (21.4)
No	329 (83.5)	83 (84.7)	86 (86.9)	82 (82.8)	78 (79.6)	85 (85.7)	87 (87.9)	80 (80.8)	77 (78.6)
History of Benign Breast Disease, n (%)ⁱ									
Yes	135 (34.4)	37 (38.1)	34 (34.3)	33 (33.3)	31 (31.6)	32 (32.6)	33 (33.7)	39 (39.4)	31 (31.6)
No	258 (65.6)	60 (61.9)	65 (65.7)	66 (66.7)	67 (68.4)	66 (67.4)	65 (66.3)	60 (60.6)	67 (68.4)
1st Degree Relative with Any Cancer, n (%)									
Yes	198 (50.3)	49 (50.0)	56 (56.6)	50 (50.5)	41 (41.8)	53 (54.1)	39 (39.4)	47 (47.5)	59 (60.2)
No	196 (49.7)	49 (50.0)	43 (43.4)	49 (49.5)	57 (58.2)	45 (45.9)	60 (60.6)	52 (52.5)	39 (39.8)
Diabetes History or Medication Use, n (%)^j									
Yes	62 (15.7)	18 (18.4)	20 (20.2)	11 (11.1)	13 (13.3)	17 (17.4)	19 (19.2)	11 (11.1)	15 (15.3)
No	209 (53.1)	67 (68.4)	53 (53.5)	42 (42.4)	47 (48.0)	53 (54.1)	38 (38.4)	57 (57.6)	61 (62.2)
Unknown	123 (31.2)	13 (13.2)	26 (26.3)	46 (46.5)	38 (38.7)	28 (28.6)	42 (42.4)	31 (31.3)	22 (22.5)
Tumor Grade, n (%)^k									
Low	50 (13.1)	9 (9.2)	10 (10.3)	15 (16.1)	16 (16.8)	11 (11.4)	7 (7.2)	13 (13.8)	19 (19.8)
Intermediate	124 (32.4)	33 (33.7)	31 (32.0)	30 (32.3)	30 (31.6)	33 (34.4)	30 (30.9)	28 (29.8)	33 (34.4)
High	209 (54.5)	56 (57.1)	56 (57.7)	48 (51.5)	49 (51.6)	52 (54.2)	60 (61.9)	53 (56.4)	44 (45.8)
AJCC Tumor Stage, n (%)^l									
I	172 (44.1)	48 (50.8)	36 (36.7)	36 (36.4)	52 (53.6)	42 (44.2)	41 (41.4)	39 (39.8)	50 (51.0)
II	167 (42.8)	38 (39.6)	46 (47.0)	48 (48.5)	35 (36.1)	45 (47.4)	41 (41.4)	44 (44.9)	37 (37.8)
III, IV	51 (31.1)	10 (10.4)	16 (16.3)	15 (15.1)	10	8	17 (17.2)	15 (15.3)	11

Characteristic	Reverse Empirical Dietary Index for Hyperinsulinemia (rEDIH) Score ^b					Reverse Empirical Dietary Inflammatory Pattern (rEDIP) Score ^b			
	Total (n = 394)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)
					(10.3)	(8.4)			(11.2)
Tumor Size (cm), n (%)^m									
< 1.0	50 (13.1)	11 (11.6)	11 (11.5)	14 (14.6)	14 (14.6)	10 (10.8)	12 (12.4)	14 (14.5)	14 (14.4)
1.0–1.9	143 (37.3)	42 (44.2)	27 (28.6)	34 (35.4)	40 (41.7)	35 (37.6)	33 (34.0)	31 (33.3)	43 (44.3)
≥ 2	190 (49.6)	42 (44.2)	58 (60.4)	48 (50.0)	42 (43.7)	48 (51.6)	52 (53.6)	50 (52.1)	40 (41.3)
Lymph Node Status, n (%)ⁿ									
Positive	137 (39.4)	29 (33.7)	35 (41.2)	44 (50.0)	29 (32.6)	26 (31.7)	41 (46.6)	36 (40.0)	34 (38.6)
Negative	211 (60.6)	57 (66.3)	50 (58.9)	44 (50.1)	60 (67.4)	56 (68.3)	47 (53.4)	54 (60.0)	54 (61.4)
Total Energy Intake (kcal/d), mean (SD)	1858 (1150)	2100 (1117)	1496 (837)	1598 (897)	2244 (1474)	2287 (1509)	1594 (793)	1494 (622)	2063 (1268)
Macronutrient Intake, mean % kcal (SD)									
Carbohydrates	48 (8.8)	45 (9.5)	47 (7.7)	50 (9.2)	52 (7.5)	49 (8.7)	49 (9.3)	47 (8.6)	49 (8.6)
Total Protein	16 (3.3)	16 (3.9)	16 (3.1)	16 (3.3)	15 (2.8)	16 (3.4)	17 (3.3)	16 (3.4)	16 (3.1)
Total Fat	36 (6.8)	39 (6.8)	37 (6.2)	35 (6.6)	34 (6.4)	37 (6.4)	35 (7.1)	38 (6.7)	36 (6.7)
Saturated Fat	11 (2.6)	12 (2.5)	11 (2.4)	10 (2.6)	10 (2.4)	11 (2.6)	10 (2.6)	11 (2.3)	11 (2.7)
Polyunsaturated Fat	8 (2.1)	9 (1.9)	9 (2.0)	8 (2.1)	8 (2.3)	8 (1.7)	8 (2.2)	9 (2.2)	8 (2.1)
Monounsaturated Fats	14 (3.1)	15 (3.1)	14 (2.7)	14 (3.8)	13 (3.0)	14 (3.0)	14 (3.1)	15 (3.2)	14 (3.0)
Total Trans Fats	2 (0.7)	2 (0.6)	2 (0.6)	2 (0.7)	2 (0.8)	2 (0.6)	2 (0.7)	2 (0.7)	2 (0.7)
Micronutrient Intake, mean per 1,000kcal (SD)									
Total Fiber (g)	9.8 (3.5)	7.6 (2.5)	9.6 (3.0)	10.2 (3.2)	11.7 (3.7)	8.5 (3.3)	9.6 (3.5)	10.0 (2.9)	11.0 (3.7)
Total Sodium (mg)	1593 (281)	1607 (304)	1622 (279)	1566 (253)	1577 (284)	1594 (322)	1603 (250)	1585 (240)	1590 (307)
a – Percentages may be greater or less than 100% due to rounding									
b – rEDIH, rEDIP, and AHEI-2010 scores were adjusted for total energy intake using the residual method. Lower scores for rEDIH represent an anti-hyperinsulinemic diet. Lower scores for rEDIP represent a pro-inflammatory diet while higher scores represent AHEI-2010 represent lower adherence to the recommendations in the scientific report for the Dietary Guidelines for Americans (2010–adherence to the recommendations in the scientific report for the dietary guidelines.									
c – Missing history or oral contraceptive use from one participant									
d – Mean age of the 219 participants who were menopausal									
e – Menopause caused by reproductive surgery (i.e. uterus or ovary removal) or medication									
f – Missing history of hormone replacement therapy use from four participants									

Characteristic	Reverse Empirical Dietary Index for Hyperinsulinemia (rEDIH) Score ^b				Reverse Empirical Dietary Inflammatory Pattern (rEDIP) Score ^b				
	Total (n = 394)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)
g – Defined as having consumed an alcoholic beverage at least once a week for six months									
h – Individuals were classified as having sufficient (150 minutes/week for moderate PA or 75 minutes/week for vigorous PA), insufficient minutes/week or 75 minutes/per), or no PA based on the aerobic component of the 2008 Physical Activity Guidelines for Americans by Prevention.									
i – Missing history of benign breast disease from 1 participant									
j – Missing responses from 122 participants – WCHS I did not ask about diabetes history; however, information about the use of diabe information on participants' history of diabetes (yes/no), as well as the method of diabetes management (oral medication or injections diabetes medication in WCHS I and any history of or medication for diabetes in WCHS II were classified as having a history of diabetes as no, and missing information classified as unknown. k – Missing tumor grade at diagnosis from 11 participants									
l – Missing tumor stage at diagnosis from 4 participants									
m – Missing tumor size diagnosis from 11 participants									
n – Missing lymph node involvement results from 46 participants									

rEDIH and IHC Marker Expression and Mortality

Each 1-SD increment in rEDIH was associated with borderline lower odds of positive nuclear expression of p-AKT (OR: 0.83; 95% CI: 0.66, 1.05) with a p-trend of 0.003 and significantly lower odds of p-P70S6K (OR: 0.76; 95% CI: 0.58, 0.98) (Table 2). A 1-SD increment in rEDIH was also associated with a borderline-significant reduction in nuclear p-P70S6K log H-score (-22.77%; 95% CI: -47.61%, 2.07%) (Supplementary Table 1). No trends were observed between rEDIH and BrCa subtype in the distribution (Supplementary Table 2).

Table 2
Multivariable-Adjusted Prevalence Odds Ratios for the Association Between Dietary Patterns and Immunohistochemical Biomarkers in Breast Tumor
Tissues^{a,b}

Dietary pattern	Statistical model	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)	P-value for linear trend ^c	1-SD Increment	P-value for continuous dietary score ^d
MTOR Cytoplasm Staining								
rEDIH	positive/negative ^e	97/1	96/3	92/7	93/5			
	MV	1 (reference)	0.59 (0.14, 2.53)	0.74 (0.16, 3.41)	0.74 (0.16, 3.41)	0.92	0.94 (0.54, 1.65)	0.84
	positive/negative ^f	96/2	98/1	90/9	94/4			
	MV + BMI	1 (reference)	0.48 (0.12, 2.00)	1.00 (0.20, 5.08)	0.74 (0.16, 3.41)	0.68	0.95 (0.55, 1.65)	0.86
rEDIP	positive/negative ^e	95/3	93/6	95/4	95/3			
	MV	1 (reference)	0.55 (0.16, 1.95)	1.35 (0.29, 6.19)	2.04 (0.37, 11.42)	0.34	1.14 (0.69, 1.86)	0.62
	positive/negative ^f	95/3	93/6	95/4	95/3			
	MV + BMI	1 (reference)	0.36 (0.09, 1.38)	1.00 (0.20, 5.08)	1.52 (0.25, 9.28)	0.16	1.14 (0.70, 1.87)	0.61
AHEI	positive/negative ^e	91/7	96/3	97/2	94/4			
	MV	1 (reference)	2.07 (0.50, 8.51)	1.53 (0.42, 5.61)	2.07 (0.50, 8.51)	0.68	1.24 (0.75, 2.04)	0.41
	positive/negative ^f	91/7	96/3	97/2	94/4			
	MV + BMI	1 (reference)	2.07 (0.50, 8.51)	1.53 (0.42, 5.61)	2.07 (0.50, 8.51)	0.68	1.24 (0.75, 2.05)	0.39
p-MTOR Cytoplasmic Staining								
rEDIH	positive/negative ^e	63/35	57/42	60/39	66/32			
	MV	1 (reference)	0.92 (5.1, 1.64)	0.68 (0.38, 1.21)	1.26 (0.69, 2.29)	0.22	0.96 (0.78, 1.91)	0.74
	positive/negative ^f	63/35	59/40	54/45	70/28			
	MV + BMI	1 (reference)	0.88 (0.49, 1.57)	0.88 (0.49, 1.57)	1.20 (0.66, 2.16)	0.69	0.99 (0.81, 1.22)	0.92
rEDIP	positive/negative ^e	59/39	54/45	61/38	72/26			
	MV	1 (reference)	0.68 (0.34, 1.21)	0.96 (0.54, 1.71)	1.45 (0.80, 2.66)	0.10	1.05 (0.85, 1.29)	0.65
	positive/negative ^f	59/39	53/46	59/40	75/23			
	MV + BMI	1 (reference)	0.65 (0.37, 1.16)	0.96 (0.54, 1.71)	1.53 (0.84, 2.80)	0.05	1.06 (0.86, 1.31)	0.58
AHEI	positive/negative ^e	56/42	63/36	63/36	64/34			
	MV	1 (reference)	0.92 (0.52, 1.63)	1.14 (0.64, 2.05)	1.05 (0.58, 1.87)	0.90	1.08 (0.88, 1.33)	0.46
	positive/negative ^f	57/41	62/37	62/37	65/33			
	MV + BMI	1 (reference)	0.88 (0.49, 1.56)	1.14 (0.64, 2.05)	1.09 (0.61, 1.96)	0.82	1.10 (0.84, 1.35)	0.37
p-AKT Cytoplasmic Staining								
rEDIH	positive/negative ^e	42/56	28/71	24/75	41/57			
	MV	1 (reference)	0.55 (0.30, 1.00)	0.67 (0.37, 1.21)	0.84 (0.47, 1.50)	0.22	0.97 (0.79, 1.19)	0.77
	positive/negative ^f	40/58	28/71	28/71	39/59			
	MV + BMI	1 (reference)	0.70 (0.38, 1.26)	0.70 (0.38, 1.26)	0.92 (0.51, 1.64)	0.51	0.96 (0.79, 1.18)	0.72

Dietary pattern	Statistical model	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)	P-value for linear trend ^c	1-SD Increment	P-value for continuous dietary score ^d
MTOR Cytoplasm Staining								
rEDIP	positive/negative ^e	43/55	24/75	24/75	44/54			
	MV	1 (reference)	0.40 (0.22, 0.75)	0.53 (0.29, 0.96)	1.13 (0.64, 2.00)	0.002	0.92 (0.75, 1.14)	0.46
	positive/negative ^f	43/55	23/76	26/73	43/55			
	MV + BMI	1 (reference)	0.36 (0.19, 0.68)	0.53 (0.29, 0.96)	1.04 (0.59, 1.84)	0.002	0.92 (0.74, 1.14)	0.44
AHEI	positive/negative ^e	36/62	29/70	32/67	38/60			
	MV	1 (reference)	0.51 (0.28, 0.95)	0.88 (0.49, 1.57)	0.96 (0.54, 1.71)	0.13	0.99 (0.80, 1.22)	0.93
	positive/negative ^f	37/61	28/71	32/67	38/60			
	MV + BMI	1 (reference)	0.57 (0.31, 1.04)	0.80 (0.45, 1.44)	0.96 (0.54, 1.71)	0.26	0.99 (0.80, 1.22)	0.90
p-AKT Nuclear Staining								
rEDIH	positive/negative ^e	57/41	41/58	30/69	55/43			
	MV	1 (reference)	0.42 (0.23, 0.74)	0.40 (0.22, 0.71)	0.75 (0.42, 1.32)	0.003	0.83 (0.66, 1.05)	0.13
	positive/negative ^f	58/40	36/63	35/64	54/44			
	MV + BMI	1 (reference)	0.47 (0.27, 0.84)	0.41 (0.23, 0.74)	0.75 (0.42, 1.32)	0.009	0.83 (0.66, 1.05)	0.13
rEDIP	positive/negative ^e	55/43	32/67	34/65	62/36			
	MV	1 (reference)	0.32 (0.18, 0.59)	0.45 (0.25, 0.80)	1.62 (0.90, 2.90)	< 0.0001	0.98 (0.80, 1.20)	0.85
	positive/negative ^f	55/43	31/68	35/64	62/36			
	MV + BMI	1 (reference)	0.25 (0.14, 0.47)	0.45 (0.25, 0.80)	1.42 (0.79, 2.54)	< 0.0001	0.98 (0.80, 1.20)	0.85
AHEI	positive/negative ^e	47/51	40/59	43/56	53/45			
	MV	1 (reference)	0.56 (0.31, 0.98)	0.88 (0.50, 1.55)	0.96 (0.55, 1.69)	0.16	1.03 (0.85, 1.26)	0.74
	positive/negative ^f	46/52	41/58	43/56	53/45			
	MV + BMI	1 (reference)	0.58 (0.33, 1.03)	0.75 (0.43, 1.32)	0.92 (0.52, 1.62)	0.25	1.04 (0.85, 1.26)	0.73
p-p70S6K Cytoplasmic Staining								
rEDIH	positive/negative ^e	55/43	45/54	40/59	52/46			
	MV	1 (reference)	0.56 (0.32, 0.99)	0.69 (0.39, 1.21)	0.72 (0.41, 1.26)	0.25	0.90 (0.72, 1.11)	0.32
	positive/negative ^f	56/42	40/59	47/52	49/49			
	MV + BMI	1 (reference)	0.69 (0.39, 1.21)	0.78 (0.44, 1.37)	0.72 (0.41, 1.26)	0.57	0.89 (0.72, 1.11)	0.30
rEDIP	positive/negative ^e	53/45	41/58	37/62	61/37			
	MV	1 (reference)	0.47 (0.26, 0.83)	0.66 (0.38, 1.16)	1.47 (0.23, 2.60)	0.0008	0.98 (0.80, 1.20)	0.84
	positive/negative ^f	54/44	38/61	38/61	62/36			
	MV + BMI	1 (reference)	0.43 (0.24, 0.77)	0.58 (0.33, 1.03)	1.29 (0.72, 2.29)	0.0007	0.98 (0.80, 1.19)	0.83
AHEI	positive/negative ^e	46/52	49/50	45/54	52/46			

Dietary pattern	Statistical model	Quartile 1 (n = 98)	Quartile 2 (n = 99)	Quartile 3 (n = 99)	Quartile 4 (n = 98)	P-value for linear trend ^c	1-SD Increment	P-value for continuous dietary score ^d
MTOR Cytoplasm Staining								
	MV	1 (reference)	0.66 (0.37, 1.16)	1.23 (0.70, 2.16)	0.92 (0.52, 1.62)	0.19	0.98 (0.80, 1.19)	0.81
	positive/negative ^f	48/50	47/52	45/54	52/46			
	MV + BMI	1 (reference)	0.72 (0.41, 1.26)	1.13 (0.64, 1.99)	0.92 (0.52, 1.62)	0.45	0.97 (0.80, 1.19)	0.79
p-p70S6K Nuclear Staining								
rEDIH	positive/negative ^e	44/54	32/67	22/77	36/62			
	MV	1 (reference)	0.51 (0.29, 0.93)	0.51 (0.29, 0.93)	0.57 (0.32, 1.02)	0.07	0.76 (0.59, 0.98)	0.03
	positive/negative ^f	47/51	27/72	25/74	35/63			
	MV + BMI	1 (reference)	0.54 (0.30, 0.97)	0.56 (0.31, 1.01)	0.59 (0.33, 1.06)	0.12	0.76 (0.58, 0.98)	0.03
rEDIP	positive/negative ^e	35/63	31/68	20/79	48/50			
	MV	1 (reference)	0.64 (0.35, 1.19)	0.68 (0.37, 1.25)	1.82 (1.02, 3.32)	0.002	1.00 (0.81, 1.23)	0.97
	positive/negative ^f	36/62	28/71	20/79	50/48			
	MV + BMI	1 (reference)	0.53 (0.29, 0.99)	0.56 (0.31, 1.04)	1.52 (0.86, 2.70)	0.001	1.00 (0.81, 1.23)	0.97
AHEI	positive/negative ^e	30/68	37/62	30/69	37/61			
	MV	1 (reference)	0.83 (0.46, 1.51)	1.05 (0.58, 1.88)	1.00 (0.55, 1.80)	0.88	1.01 (0.82, 1.24)	0.94
	positive/negative ^f	32/66	35/64	30/69	37/61			
	MV + BMI	1 (reference)	0.87 (0.48, 1.58)	1.00 (0.55, 1.80)	1.00 (0.55, 1.80)	0.96	1.01 (0.82, 1.24)	0.95
a - Adjusted for age, total energy intake, waist-hip ratio, education, age at menarche and menopause, oral contraceptive and hormone replacement therapy use, parity, breastfeeding, reproductive years, smoking, alcohol use, benign breast disease history, family history of BrCa and cancer, recreational physical activity, and diabetes history..								
b - Adjusted for MV adjustments plus BMI								
c - The p-value for linear trend was estimated in the same multivariable-adjusted models by assigning the quartile-specific median value of each dietary pattern to all participants in the quartile and modeling as an ordinal variable.								
d - Dietary score was input in the corresponding multivariable-adjusted models as a continuous variable								
e - Multivariable-adjusted prevalence odds ratio								
f - Multivariable plus BMI adjusted prevalence odds ratio								

In multivariable-adjusted Cox regression, each 1-SD increment in rEDIH was associated with lower risk of all-cause (HR: 0.84; 95% CI: 0.73, 0.95) and BrCa-specific mortality (HR: 0.81; 95% CI: 0.72, 0.93). While slightly attenuated, trends in hazard ratios from Cox regression sensitivity analysis remained consistent with the multivariable-adjusted models with each 1-SD increment in rEDIH being associated with lower risk of all-cause mortality (HR: 0.87; 95% CI: 0.74, 1.01) and BrCa-specific mortality (HR: 0.84; 95% CI: 0.73, 0.96) (Supplementary Table 3).

In stratified analyses by IHC marker status, higher rEDIH was associated with lower all-cause mortality risk among women with p-MTOR-positive (cytoplasmic; HR: 0.82; 95% CI: 0.71, 0.95), p-AKT-positive (nuclear), and p-P70S6K-positive (nuclear) tumors (Fig. 1A). In stratified sensitivity analysis by IHC marker status, associations of significance between higher rEDIH and mortality risk were slightly attenuated with lower all-cause mortality risk among women with p-P70S6K positive cytoplasmic and nuclear tumors no longer of statistical significance (results not shown). For BrCa-specific mortality, higher rEDIH was associated with lower risk among women with tumors positively expressing cytoplasmic MTOR, cytoplasmic p-MTOR, nuclear p-AKT, cytoplasmic p-P70S6K, and nuclear p-P70S6K (e.g., HR: 0.81; 95% CI: 0.69, 0.95 for nuclear p-P70S6K), as well as among women with tumors negatively expressing cytoplasmic p-AKT, cytoplasmic p-P70S6K, and nuclear p-P70S6K (e.g., HR: 0.62; 95% CI: 0.41, 0.93 for nuclear p-P70S6K) (Fig. 1B). For stratified sensitivity analysis by IHC marker status, the association between higher rEDIH and mortality risk were attenuated with lower BrCa-specific mortality risk among women with p-P70S6K positive cytoplasmic tumors no longer of statistical significance (results not shown).

rEDIP and IHC Marker Expression and Mortality

Higher rEDIP was not associated with IHC marker expression irrespective of staining location (e.g., OR: 1.00; 95% CI: 0.81, 1.23 for nuclear p-P70S6K; OR: 1.06; 95% CI: 0.86, 1.31 for cytoplasmic p-MTOR) (Table 2), nor with overall all-cause or BrCa-specific mortality, nor all-cause or BrCa-specific mortality from sensitivity analysis (Supplementary Table 3). In marker-stratified analyses, borderline-significant associations were observed between higher rEDIP and lower all-cause mortality among women with nuclear p-AKT- and nuclear p-P70S6K-positive tumors (e.g., HR: 0.74; 95% CI: 0.54, 1.02 for nuclear p-P70S6K) (Fig. 2A). Higher rEDIP was associated with lower BrCa-specific mortality among women with tumors positively expressing nuclear p-AKT, cytoplasmic p-P70S6K, and nuclear p-P70S6K (e.g., HR: 0.66; 95% CI: 0.47, 0.92 for nuclear p-P70S6K) (Fig. 2B). The reduction in BrCa-specific mortality risk among women positively expressing p-AKT and p-P70S6K in the nucleus was attenuated and not of statistical significance in sensitivity analysis (results not shown).

AHEI-2010 and IHC Marker Expression and Mortality

Higher AHEI-2010 was not associated with IHC marker expression in either cytoplasmic or nuclear staining (e.g., OR: 1.01; 95% CI: 0.82, 1.24 for nuclear p-P70S6K; OR: 0.99; 95% CI: 0.80, 1.22 for cytoplasmic p-AKT) (Table 2). A notable exception was a positive association between AHEI-2010 and cytoplasmic MTOR log H-score (12.39%; 95% CI: 1.13%, 23.64% for 1-SD increment), indicating higher MTOR protein expression with better overall diet quality (Supplementary Table 1). No associations were found between AHEI-2010 and all-cause mortality (Fig. 3A), or BrCa-specific mortality (Fig. 3B) nor all-cause and BrCa-specific mortality in sensitivity analysis (Supplementary Table 3).

Sensitivity Analyses Results

Results from the sensitivity analysis using NDSR-standardized portion sizes were consistent in direction with the primary results (Supplementary Tables 4–5 and Supplementary Figs. 1–3). For rEDIH, nuclear p-P70S6K remained the most consistently associated IHC marker (OR: 0.66; 95% CI: 0.48, 0.91 for 1-SD increment; Supplemental Table 5), with a similar but slightly attenuated estimate compared to the primary analysis (OR: 0.76; 95% CI: 0.58, 0.98). The standardized method also yielded a borderline-significant inverse association between rEDIH and cytoplasmic p-MTOR (OR: 0.73; 95% CI: 0.52, 1.03; $p = 0.06$) that was not evident in the primary analysis. For rEDIP, both methods showed significant inverse associations with nuclear p-AKT across quartiles (p -trend = 0.0006 for the standardized method vs. $p < 0.0001$ for the primary method), suggesting this finding is robust. Associations with cytoplasmic p-MTOR were stronger under the standardized method (p -trend = 0.03) than the primary method, where this association was not statistically significant. For survival analyses, notably, a higher rEDIH was associated with lower mortality among women with p-MTOR, p-AKT, and p-P70S6K positive cancers with borderline significance, but not among those with negativity of these tumor markers (Supplemental Fig. 1).

DISCUSSION

In this study of Black women with BrCa, we examined associations between dietary patterns reflecting low insulinemic potential (rEDIH), low inflammatory potential (rEDIP), and overall dietary quality (AHEI-2010) in relation to tumor expression of PI3K/AKT/MTOR pathway markers and BrCa mortality. Our findings suggest that low-insulinemic dietary patterns are associated with lower odds of tumor positivity for key pathway markers and with lower mortality risk among women whose tumors express these markers. Associations were more limited for rEDIP and absent for AHEI-2010, suggesting that mechanistically targeted dietary indices may better capture biologically relevant processes in BrCa progression than global dietary quality scores.

Although several associations were of borderline statistical significance, effect sizes are clinically meaningful in a population with historically elevated BrCa incidence and mortality. Each 1-SD increment in rEDIH was associated with 17–24% lower odds of positive expression of PI3K/AKT/MTOR pathway markers and a 19–22% reduction in BrCa-specific mortality among women with tumors positively expressing cytoplasmic MTOR, p-MTOR, cytoplasmic and nuclear p-P70S6K, and nuclear p-AKT. These findings are consistent with emerging evidence linking diet-related insulin and inflammatory signaling to tumor behavior and outcomes.^{10,36} Differences in associations across dietary indices underscore the value of metabolically targeted exposure measures over broad dietary quality scores. The positive association between AHEI-2010 and cytoplasmic MTOR H-score, suggesting higher MTOR protein expression with better overall diet quality, may reflect residual confounding and should be interpreted cautiously pending replication.

MTOR is a serine/threonine kinase that integrates signals from growth factors, nutrients, and energy status to regulate cell growth, proliferation, and survival. Its hyperactivation drives lipid biosynthesis and anabolic processes associated with poor prognosis, therapeutic resistance, and aggressive tumor phenotypes.^{37–40} Phosphorylated MTOR acts downstream of PI3K/AKT signaling as a critical oncogenic effector. Nuclear p-AKT promotes cell survival by regulating transcription factors, DNA repair proteins, and apoptotic regulators, with nuclear localization reflecting sustained pathologic activation and genomic instability.^{23,41,42} p-P70S6K, a direct downstream target of MTORC1, completes a critical oncogenic axis with p-MTOR and nuclear p-AKT.^{43–46} Their coordinated activation, particularly in the context of hyperinsulinemic or pro-inflammatory dietary patterns, may drive carcinogenic processes and represent actionable therapeutic targets, as PI3K, AKT, and MTOR inhibitors are in active clinical development or use.^{47,48} Our finding that higher rEDIH is consistently associated with lower mortality risk, particularly among Black women with tumors expressing markers of an overactivated PI3K/AKT/MTOR pathway, positions rEDIH as a potentially important dietary modulator of aggressive tumor behavior and a candidate adjuvant to anti-cancer treatment regimens.

To our knowledge, this is the first study examining the associations between insulinemic and inflammatory dietary patterns, the PI3K/AKT/MTOR pathway, and BrCa outcomes. Prior work is limited to single foods or nutrients: pre-diagnostic coffee intake was weakly associated with PI3K activation score and markers of angiogenesis but not with PTEN loss or insulin-related receptors;⁴⁹ other studies have examined green tea polyphenols in gynecological cancer,⁵⁰ anthocyanins and obesity,⁵¹ vitamin K2 and AKT/MTOR signaling,⁵² and dietary insulinemic indices and PI3K signaling in adipose tissue.⁵³ The current study extends this literature by examining habitual dietary patterns rather than single dietary components, in relation to a panel of pathway markers and survival outcomes. The consistent direction of findings toward reduced mortality with higher rEDIH among Black women is particularly noteworthy given documented racial disparities in both dietary insulinemic exposure and BrCa-specific mortality. These results suggest that modifiable dietary behavior may partially mediate survival disparities and support targeted dietary intervention in this high-risk population.

The strengths of this study include detailed dietary data, IHC-quantified tumor tissue markers, comprehensive BrCa risk factor data, and mortality follow-up in a cohort of Black women, with H-scores derived from automated digital image analysis, a detailed sensitivity analysis demonstrating the robustness of our findings. Limitations of this study include the relatively small sample size, which precluded quartile-level mortality analyses and evaluation of clinically meaningful dietary intake thresholds. The FFQs, though well-validated, are truncated instruments less suited to capturing full dietary variation. Assessment of PI3K/AKT/MTOR pathway activation was narrow, limited to one marker upstream (p-AKT) and one downstream (p-P70S6K) of MTOR, with no data on IGF-1 receptor or PTEN. Finally, the absence of a standard definition for MTOR marker positivity limits comparability across studies.^{14,15}

CONCLUSION

Low-insulinemic dietary patterns, as captured by rEDIH, are associated with lower BrCa-specific mortality in Black women through modulation of the PI3K/AKT/MTOR pathway. These findings reinforce the potential of pathway-targeted dietary indices to identify individuals at elevated risk for poor outcomes and to inform precision nutrition strategies, as well as highlight the importance of using mechanistically specific dietary instruments in cancer research. Larger prospective studies in racially diverse populations are needed to confirm these findings and further clarify the biological mechanisms linking dietary patterns to tumor pathway activity and survival among patients with breast cancer. High-rEDIH dietary patterns warrant evaluation as clinical trial interventions, particularly among women with BrCa and evidence of tumor PI3K/AKT/MTOR overactivation.

DATA AVAILABILITY

The data supporting the findings of this study are not publicly available to protect patient privacy. The data will be made available to authorized researchers with the approval of the Women's Circle of Health Study (WCHS) committee and relevant Institutional Review Boards.

CODE AVAILABIILY

The underlying code for this study is not publicly available but may be made available to qualified researchers upon reasonable request from the corresponding author.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol was approved by all relevant institutional review boards, including Roswell Park comprehensive Cancer Center and Rutgers Cancer Institute of New Jersey. All participants provided written informed consent

CONFLICT OF INTEREST

Dr. Bandera is supported by the Rutgers Unilever Endowed Chair in Nutrition and Cancer Prevention and served on a Pfizer Inc. Advisory Board to enhance minority participation in clinical trials, both unrelated to this work. All authors claimed no direct conflict of interest related to the submitted work.

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Author Contribution

Study conception and design: F.K.T. and T.D.C. Funding: T.D.C., E.V.B, and B.Q. Data acquisition: B.Q., E.V.B., T.W., A.R.O, S.Y., C.C.H, T.K., and T.D.C. Writing – initial draft: A.E.K. Data analysis: A.E.K. Data interpretation: A.E.K and F.K.T. Technical review: E.O. O Writing – final review and approval: B.Q., A.H., X.F.S.Z, E.V.B., T.W., S.Y., A.R.O., C.C.H., T.K., T.D.C, and F.K.T.

Data Availability

The data supporting the findings of this study are not publicly available to protect patient privacy. The data will be made available to authorized researchers with the approval of the Women's Circle of Health Study (WCHS) committee and relevant Institutional Review Boards.

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Figures

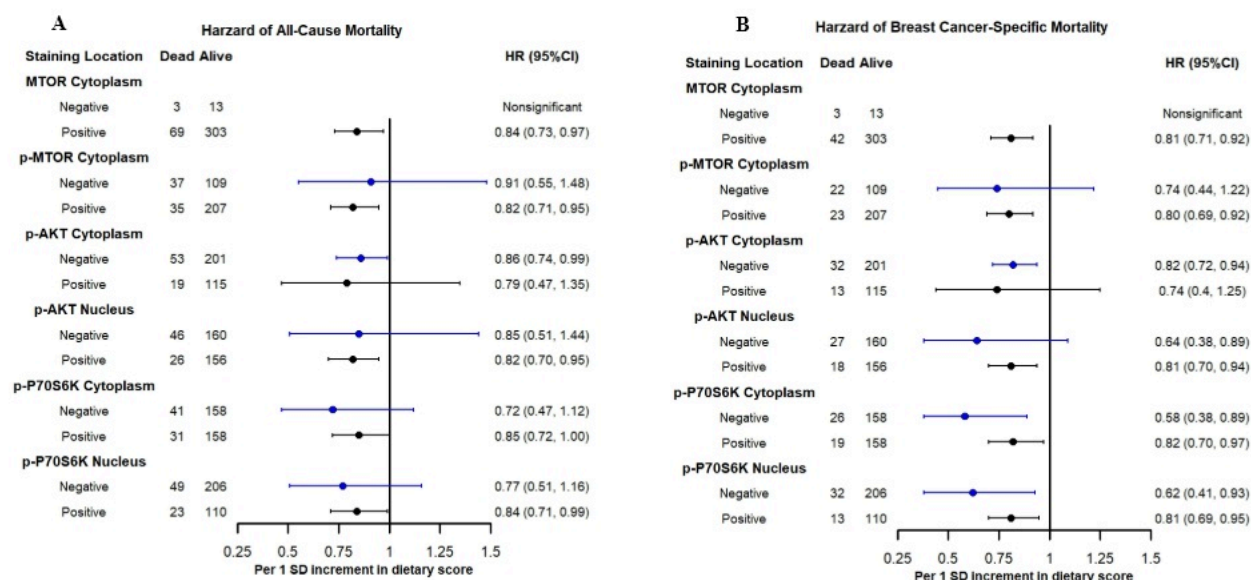


Figure 1

A. Multivariable-adjusted (MV)-adjusted association between rEDIH and all-cause mortality by expression of markers along PI3K/AKT/MTOR pathway; **B.** MV-adjusted association between rEDIH and breast cancer-specific mortality by expression of markers along PI3K/AKT/MTOR pathway. Hazard ratios were derived from Cox proportional hazard analysis adjusted age, total energy intake, waist-hip ratio, education, age at menarche and menopause, oral contraceptive and hormone replacement therapy use, parity, breastfeeding, reproductive years, smoking, alcohol use, benign breast disease history, family history of BrCa and cancer, recreational physical activity, diabetes history and BMI.

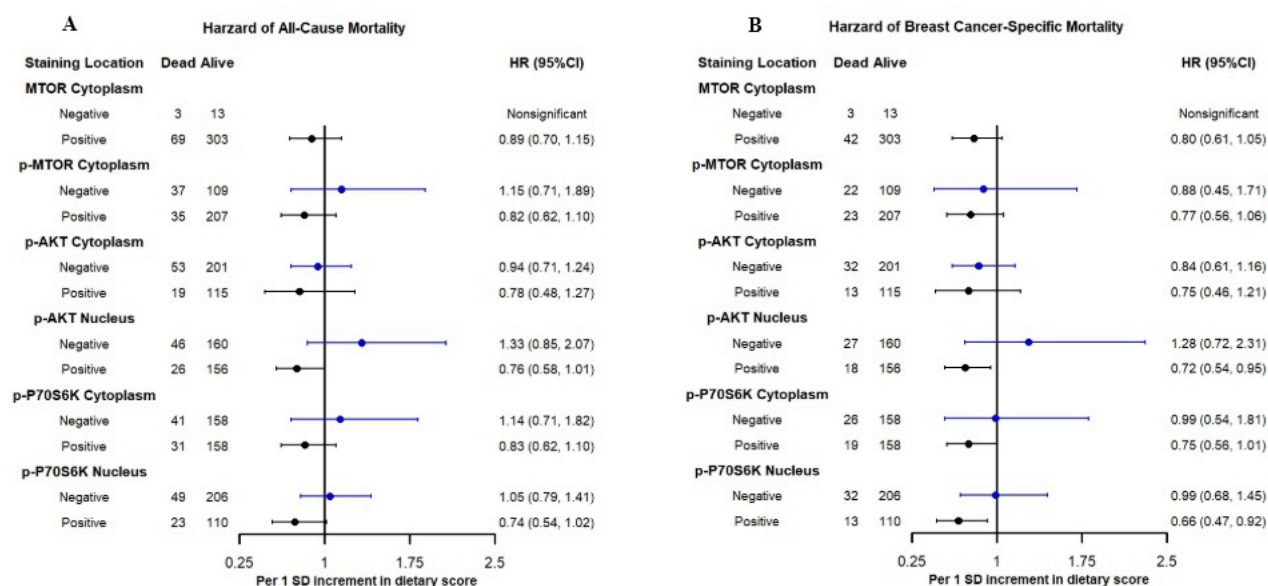


Figure 2

A. Multivariable-adjusted (MV)-adjusted association between rEDIP and all-cause mortality by expression of markers along PI3K/AKT/MTOR pathway; **B.** MV-adjusted association between rEDIP and breast cancer-specific mortality by expression of markers along PI3K/AKT/MTOR pathway. Hazard ratios were derived from Cox proportional hazard analysis adjusted age, total energy intake, waist-hip ratio, education, age at menarche and menopause, oral

contraceptive and hormone replacement therapy use, parity, breastfeeding, reproductive years, smoking, alcohol use, benign breast disease history, family history of BrCa and cancer, recreational physical activity, diabetes history and BMI.

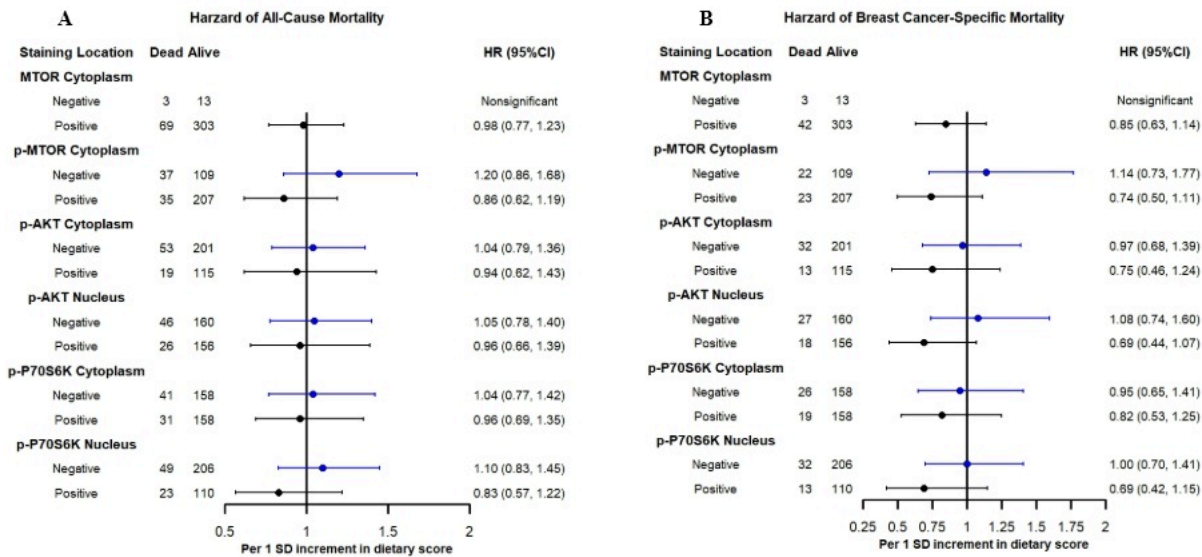


Figure 3

A. Multivariable-adjusted (MV)-adjusted association between AHEI-2010 and all-cause mortality by expression of markers along PI3K/AKT/MTOR pathway; **B.** MV-adjusted association between AHEI-2010 and breast cancer-specific mortality by expression of markers along PI3K/AKT/MTOR pathway. Hazard ratios were derived from Cox proportional hazard analysis adjusted for age, total energy intake, waist-hip ratio, education, age at menarche and menopause, oral contraceptive and hormone replacement therapy use, parity, breastfeeding, reproductive years, smoking, alcohol use, benign breast disease history, family history of BrCa and cancer, recreational physical activity, diabetes history and BMI. .

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