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RESEARCH

The Effects of Protein Restricted Diet and Physical Activity on Preoperative Tumor Characteristics and Progression in Stage I-II Breast Cancer Cases: A Randomized Clinical Trial

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Abstract

Introduction This study investigated short-term changes in tumor characteristics and progression in patients with early-stage breast cancer between diagnosis and surgery using a protein-restricted diet and physical exercise intervention.

Materials and methods Eighty patients diagnosed with breast cancer and clinically stage I-II at study entry were divided into two groups. The intervention (study) group were given an isocaloric protein-restricted (7%) diet and 10,000-step walking exercise every three days. The patients in the control group were also given an isocaloric diet but protein content was 21% and they did not undergo exercise intervention. Serum biomarkers, including Insulin Like Growth Factor-1 (IGF-1), Tumor Necrosis Factor alpha (TNFα), Fibroblast Growth Factor (FGF), Vascular Endothelial Growth Factor (VEGF), insulin, and sex hormones were measured in all participants on the morning of the first day of the diet and the morning of the surgery day; the duration between these two time points was approximately 14 days in both groups. In addition, tumor diameters and histopathological Ki-67 expression ratios were compared before and after the diet.

Results The results were compared separately within the ER (+), PR (+), HER2 (-), Luminal A, and Luminal B HER2 (-) subgroups. The tumor diameters and Ki-67 ratios of patients in the control group had increased in the two weeks before surgery. However, there was no significant increase in tumor diameter or Ki-67 ratio in the study group over the same durtion. Furthermore, in the study group there was a notable decrease in the levels of IGF-1, TNFα, and VEGF.

Conclusion A prolonged delay from diagnosis to surgery in patients with breast cancer may adversely affect prognosis. In situations such as the COVID-19 pandemic, preoperative herpes labialis infection, and upper respiratory tract infection, when delay may occur, introducing a protein-restricted diet with additional physical activity may mitigate the negative impact on prognosis.

Keywords Breast tumor · Protein-restricted diet · Ki-67 · IGF-1 · VEGF

1 Introduction

Among women, breast cancer is the most common malignancy worldwide and one of the most frequent reasons for death [1]. The prognosis for breast cancer differs depending on patient and tumor characteristics. Preclinical studies have suggested that a negative energy balance slows down cancer progression, potentially by reducing inflammation and altering hormone levels that contribute to tumor growth. Inflammatory processes involving signal pathways, insulin, insulin-like growth factor-1 (IGF-1), sex hormones, and sex hormone-binding globulin (SHBG) link obesity and cancer [2, 3].

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Some studies have suggested that environmental factors play an important role in breast cancer pathogenesis. The Western diet with high protein content has been associated with an increased incidence of breast cancer [4]. However, data from previous epidemiological and experimental trials show that IGF-1 levels in circulation activate the Akt (protein kinase B)/mTOR pathway, and the high levels of dietary protein have been linked to activating this pathogenetic mechanism [5].

Furthermore, elevated circulating levels of IGF-1 are associated with an increased risk of breast cancer [6–8]. In a study conducted using human xenograft animal models, a comparison was made by implementing isocaloric diets with 7% and 21% protein levels [9]. It was demonstrated that the group with the 7% isocaloric protein restriction exhibited a 56% reduction in tumor size compared to the group receiving a 21% protein diet. The study demonstrated differential growth of human WHIM16 tumors in mice on a high-protein diet compared to significant inhibition of tumor growth by low-protein consumption. It was postulated that the observed decreased tumor progression may have occurred because of inhibition of the IGF/Akt/mTOR pathway, and the lower Ki-67 mitosis index found in low-protein diet animals [9].

The COVID-19 pandemic resulted in a delay in diagnosing and treating cancer cases which may have had a negative impact on patient outcomes. The present study aimed to examine if a low-protein diet and increased physical exercise before surgery affected tumor growth in human breast cancer patients as a response to the delays in diagnosing and treating cancer cases during the COVID-19 pandemic and which may occur again in the future.

2 Materials and Methods

2.1 Patients and Study

Between May 2021 and March 2022, a population-based cohort of 80 operable stage I and stage II breast cancer patients was randomly assigned into a study group with restricted protein consumption with planned increased physical activity ($n=40$) or a control group that received the standard preoperative nutrition and applied routine with no change to their ordinary physical activity levels ($n=40$). The study was an open-label study for the patients and physicians, except for all laboratory-based researchers. This investigator-initiated study was approved by the Regional Ethics Committee for Research Funding at Kocaeli University (Approval No.: TTU-2022-2822, Date: 01/03/2022); however, it was not prospectively registered in a public trial registry.

The overall study sample was modest, and the trial was an exploratory, randomized study focused on short-term biological and metabolic changes during the preoperative interval. Pathological diagnosis of breast cancer was performed using conventional histopathological techniques. The main biological interpretation was based primarily on intrinsic molecular subtype categories, particularly Luminal A and Luminal B HER2-negative disease, rather than on ER-positive, PR-positive, or HER2-negative status alone. Receptor-based analyses were secondary, supportive, and exploratory. The rationale for this decision was that receptor-only subgrouping may pool biologically distinct tumors and thereby obscure subtype-specific patterns. The receptor-based subtype, subgroup analyses were not powered for definitive inference and should therefore be considered hypothesis-generating rather than confirmatory. Furthermore, the study may be underpowered for such analysis and the probability of Type I error is increased due to the limited sample size and multiple assessed parameters. No a priori sample size calculation based on a predefined primary endpoint was performed; thus, statistical inference should be interpreted cautiously. Sample size estimation was performed using G*Power software; however, due to the exploratory nature of the study, no single primary endpoint–based formal power calculation was predefined. G*Power analysis showed that eighty patients should be recruited to the study. These patients would be newly diagnosed stage I or II breast cancer patients without any previous restricted diet or exercise. The exclusion criteria were clinical or radiologically staged 3 or 4 tumors, ductal carcinoma in situ (DCIS), being younger than 18 or older than 65 years of age, being unable to cooperate, or having comorbidities that prevented protein diet restriction and/or increased physical activity.

The study protocol was explained to all candidates, and informed signed consent was obtained from all participants. Randomization was performed following consent being obtained; allocation was conducted using sealed envelopes placed in two boxes, with balanced assignment according to age and menopausal status through 1:1 block randomization. Patient enrollment was consecutive among eligible stage I–II cases. Laboratory investigators and the histopathologist were blinded to group allocation, whereas the clinical intervention itself was open-label by design.

The principal biological efficacy outcome was the short-term change in Ki-67 between the baseline biopsy and the surgical specimen, because Ki-67 was the most direct tumor proliferation marker assessed in relation to the preoperative intervention. Tumor diameter change and circulating biomarkers were classified as secondary or exploratory outcomes. The biomarker analyses and subgroup analyses were exploratory and hypothesis-generating rather than confirmatory because of limited sub-group sizes.

A registered dietitian performed repeated 24-hour dietary assessments from enrollment to surgery. Patient energy requirements were calculated using the Schofield formula and both diets taken by the intervention and control group patients were isocaloric but differed in protein proportion (7% vs. 21%, respectively) [10]. Finally, body composition was measured using the Tanita BC-418 MA analyzer. Dietary adherence was monitored but not independently validated by additional objective nutritional biomarkers.

A registered dietitian from the endocrinology and metabolism department conducted 24-hour protein-restricted dietary assessments for each participant throughout the preoperative period from obtaining informed consent to the day of surgery, which was around 14 days in practice. The physical activity in the intervention group was monitored with an OMRON HJ-321-E pedometer and targeted at 10,000 steps every three days during the preoperative interval. Patients in the protein-restricted diet group were monitored through daily assessments and the pedometer device to ensure they engaged in light-paced walking of 10,000 steps every three days. The isocaloric diet contained 7% and 21% protein for the intervention and control groups, respectively, and balanced carbohydrates and lipids were prepared according to any patients' comorbidities.

3 Follow-Up Assessment

Body composition of all participants was measured by the Tanita BC-418 MA (BIA) body analyzer. The Anthropometric Standardization Manual used for measurement of weight and height. BMI was calculated using the standard formula and reported as kg/m² [11].

Preoperative diagnosis was performed via core biopsy. Tumor size was assessed using the same imaging modality (breast ultrasound or mammography, depending on initial visibility) at baseline and immediately prior to surgery. Measurements were performed by experienced radiologists using standardized institutional protocols. Whenever feasible, the same imaging modality and evaluator were used at both time points. Radiologists were not formally blinded to allocation although histopathological assessment, including Ki-67, was performed by a blinded pathologist. Specimens were assessed by immunohistochemistry for hormone and HER2/neu receptors. Once assessment was complete, the pathologist reported the cancer type and subgroup for each patient. After surgery, the same pathologist also assessed sufficient tumor tissue, and the same parameters were assessed, identified and documented.

4 Biochemical Analysis

Blood samples collected with venipuncture were separated into sera, plasma, and buffy coats. All separated samples were stored at -80 °C until analysis. Identical parameters were also measured from collected blood samples on the operation day.

The following assays were carried out in our institution's research laboratory. Sera were analyzed for leptin (Human Leptin ELISA Kit-BT LAB Liaxing, Zhejiang/China - Cat. No: E1559Hu), TNF-a (Human Tumor Necrosis Factor alfa Elisa Kit-BT LAB Jiaxing, Zhejiang/China - Cat.No: E0082Hu), bFGF (Human Basic Fibroblast Growth Factor Elisa Kit-BT LAB Jiaxing, Zhejiang/China - Cat.No: E0145Hu), and VEGF-C (Human Vascular Endothelial Cell Factor C Elisa Kit-BT LAB Jiaxing, Zhejiang/China - Cat. No: E0079Hu) ELISA kits at Alisei Quality System Seac Radim Company.

The levels of serum estradiol, testosterone, sex hormone-binding globulin, albumin, total protein, lipid profiles, insulin, C-peptide, IGF-1, and interleukin 6 (IL-6) were measured using chemiluminescent methods on Cobas analyzer series e801 and c702, and the Immulite 200 XPI Analyzer at Kocaeli University Hospital Routine Laboratory.

4.1 Statistical Analysis

Statistical analyses were conducted using SPSS, version 20.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Normally distributed variables are reported as mean ± standard deviation, whereas non-normally distributed variables are presented as median and interquartile range in parentheses (IQR; 25th–75th percentile). Categorical variables are expressed as frequencies and percentages. Intergroup differences were analyzed using the independent samples t-test for variables with a normal distribution and the Mann-Whitney U test for non-normally distributed variables. For paired group comparisons, the paired t-test was employed when the normality assumption was satisfied, and the Wilcoxon signed-rank test was utilized when it was not. Associations between categorical variables were evaluated using the chi-square test. Statistical significance was determined at a two-tailed p-value of < 0.05. In addition to p-values, absolute changes (Δ) between baseline and post-intervention measurements were calculated for key outcomes. Where appropriate, 95% confidence intervals (CIs) were estimated to provide effect size interpretation.

5 Results

This study was conducted between March 2021 and March 2022. All patients diagnosed with early breast cancer were intended to be recruited to the study. However, about one-third of newly diagnosed patients with breast cancer declined to consent (for various reasons, such as personal beliefs or concerns about the study), and another one-third were not eligible for the study because of comorbid disorders or tumor characteristics. This meant that the final study cohort was 80 cases enrolled in each group. Table 1 shows the demographic and physiological data for each group. Mean ages were 51.70 ± 8.93 years in the control group and 50.25 ± 10.14 years in the diet group ($p > 0.05$).

Menarche age, menopausal status, labor count, smoking habit, comorbidities such as diabetes mellitus and hypertension, and education level were similar in the two groups. Most of the cases were invasive ductal carcinoma in both groups. Most patients in both groups underwent breast-conserving surgery (Table 1). Patients enrolled in the study were operated on after 13.6 ± 1.2 days in the control group and 13.9 ± 1.6 days in the dietary group ($p > 0.05$) .

HER2-negative patients in both groups were compared. Notably, the BMI of the dietary group exhibited a significant change over the period before surgery.(Table 2) While K-i67 levels increased in both groups, the difference between diagnosis and immediately prior to surgery was only significant in the control group. Furthermore, SHBG, IL-6, and bFGF serum levels demonstrated significant changes exclusively in the control group. Moreover, total cholesterol, HDL cholesterol, albumin, and total protein levels showed a significant decline from diagnosis to surgery in both groups. However, insulin and VEGF levels significantly decreased in the dietary group ($p < 0.001$) (Table 3) (Fig. 1). When comparing the groups at baseline and immediately before surgery, statistically significant differences were observed in Ki67 ($p = 0.018$), C-peptide ($p = 0.038$), IL-6 ($p = 0.012$), and leptin levels ($p < 0.001$) on the day of the operation (Figs. 2, 3, 4 and 5). Of note, at the initiation of the dietary intervention, leptin levels were significantly lower, whereas VEGF

Table 1 Demographic data for both groups (CG: control group; DG: dietary intervention group)

		Groups	
		CG ($n = 40$)	DG ($n = 40$)
Age		51,70 ± 8,93	50,25 ± 10,14
Menarche age		13,2 ± 1,13	13,32 ± 1,28
Menopausal Status	**Premenopausalausal**	14 (%35)	19 (%47,5)
	Postmenopausal	26 (%65)	21 (%52,5)
Labor	**≤2**	27 (%67,5)	31 (%77,5)
	≥3	13 (%32,5)	9 (%22,5)
Education	**High School**	30(%75)	24(%60)
	University	10(%25)	16(%40)
Smoking	**Yes**	16(%40)	11(%27,5)
	No	24(%60)	29(%72,5)
DM	**Yes**	6(%15)	5(%12,5)
	No	34(%85)	35(%87,5)
Hypertension	**Yes**	13(%32,5)	9(%22,5))
	No	27(%67,5)	31(%77,5)
Tumor Histological Type	**Invasive ductal**	39(%97,5)	36(%90)
	Invasive lobular	0(%0)	2(%5)
	Mucinous carcinoma	1(%2,5)	2(%5)
Receptor Status	**ER positive**	35(%87,5)	38(%95)
	ER negative	5(%12,5)	2(%5)
	PR positive	29(%72,5)	38(%95)
	PR negative	11(%27,5)	2(%5)
	HER2 positive	37(%92,5)	37(%92,5)
	HER2 negative	3(%7,5)	3(%7,5)
Molecular Sub Groups	**Luminal A**	8(%20)	17(%42,5)
	Luminal B Her2-	26(%65)	19(%47,5)
	Luminal B Her2+	2(%5)	2(%5)
	Triple negative	3(%7,5)	1(%2,5)
	Non Luminal Her2+	1(%2,5)	1(%2,5)
Surgery type	**BCS+SLNB**	25(%62,5)	23(%57,5)
	BCS+SLNB+AD	12(%30)	11(%27,5)
	Mastectomy+SLNB	2(%5)	1(%2,5)
	Mastectomy+SLNB+AD	1(%2,5)	5(%12,5)

Results are shown as mean±standard deviation or n(%). Abbreviations: CG, control group; DG, dietary intervention group; ER, estrogen receptor; PR, progesterone receptor; BCS, breast-conserving surgery; SLNB, sentinel lymph node biopsy; AD, axillary disection

Table 2 BMI for patients in both groups

		HER2 (-) CG (*n*=37)	**HER2 (-) DG (*n*=37)**	***p***
	Before	29,34(25,64−33,27)	28,93(26,01–31,68)	0,867
	After	29,07(25,47−33,30)	28,47(25,60−31,21	0,791
		ER (+) CG (*n*=35)	**ER (+) DG (*n*=38)**	**p**
	Before	29,34(25,54−33,58)	28,94(25,81−32,73)	0,089
	After	29,71±5,58	29,49±5,78	0,873
		PR (+) CG (*n*=29)	**PR (+) DG (*n*=38)**	**p**
BMI (kg/m^2)	Before	29,58(25,64−34,74)	28,94(25,81−32,73)	0,714
	After	30,14±5,75	29,49±5,78	0,649
		Luminal A CG (*n*=8)	**Luminal A DG (*n*=17)**	**p**
	Before	30,43(28,26–35,81)	28,94(25,76−35,44)	0,549
	After	30,58(28,07–35,91)	28,47(25,62−35,88)	0,511
		Luminal B HER2(-)CG (*n*=20)	**Luminal B HER2(-)DG (*n*=19)**	**p**
	Before	28,31(25,44−33,79)	28,92(25,95−31,20)	0,963
	After	28,29(25,20–34,03)	28,80(25,59−31,01)	0,890

Change of BMI for DG following dietary intervention and CG HER2(-) CG $p=0.959$ DG $p=0.001$

ER(+) CG $p=0.883$. DG $p=0.001$

PR (+). CG $p=0.764$. DG $p=0.001$

Luminal A CG $p=0.123$ DG $p=0.007$

Luminal B HER2(-) CG $p=0.575$ DG $p=0.001$

Table 3 Comparisons of biochemical parameters for HER2 (-), ER(+), and PR(+) patients in control and dietary groups

Biochemical parameters				
		HER2 (-) CG (*n*=37)	**HER2 (-) DG (*n*=37)**	***p***
Ki-67(%)	Before	17,00(10,00–24,00)	14,00(9,50−25,00)	0,725
	After	22,00(16,50−32,00)	17,00(8,00–25,50)	**0,018***
C-peptide (ng/mL)	Before	3,73(2,44−4,64)	3,16(2,51−5,14)	0,795
	After	2,72(2,02–4,22)	2,34(1,88−2,78)	**0,038***
IL-6 (pg/mL)	Before	3,48(2,13−5,47)	4,07(2,04–5,56)	0,983
	After	5,77(4,13−9,01)	4,47(3,11−5,32)	**0,012***
Leptin (ng/mL)	Before	6,51(4,13−10,45)	2,44(1,76−3,61)	**<0,001***
	After	6,58(3,76−11,59)	2,58(2,09−3,60)	**<0,001***
VEGF-C (ng/L)	Before	3503,05(2662,17-4641,17)	5408,27(4060,42-6195,88)	**<0,001***
	After	4068,15(3128,56-5193,27)	3591,99(3093,35-4233,07)	0,171
		ER (+) CG (*n*=35)	**ER (+) DG (*n*=38)**	**p**
Total Protein(g/L)	Before	69,56±5,56	72,13±3,97	**0,028**
	After	65,10(61,30–67,80)	67,05(62,98−70,45)	0,235
Insulin (uIU/mL)	Before	14,40(8,86−26,60)	12,45(8,86−23,75)	0,675
	After	11,40(4,78−26,80)	7,87(4,79−11,23)	**0,016**
C-peptide (ng/mL)	Before	3,75(2,53−4,87)	3,24(2,57−5,33)	0,588
	After	3,51(2,15−4,34)	2,33(1,83−2,75)	**0,003**
IL-6 (pg/mL)	Before	3,68(2,24−5,19)	4,11(2,06−5,43)	0,907
	After	6,09(4,23−9,63)	4,40(2,72−5,32)	**0,002**
Leptin(ng/mL)	Before	6,43(3,95−9,61)	2,36(1,76−3,58)	**<0,001**
	After	6,48(3,75−10,36)	2,38(2,08−3,59)	**<0,001**
VEGF-C(ng/L)	Before	3169,97(2711,13-4570,42)	5357,65(4098,66-6183,00)	**<0,001**
	After	3850,22(3018,22-5120,49)	3580,27(3066,46-4303,51)	0,414
		PR (+) CG (*n*=29)	**PR (+) DG (*n*=38)**	**p**
SHBG (mmol/L)	Before	32,20(23,95−55,25)	40,55(29,68−59,40)	0,147
	After	33,10(21,75−47,50)	40,15(29,45–60,13)	**0,043**
Insulin (uIU/mL)	Before	19,40(9,21–29,75)	12,45(8,86−23,75)	0,404
	After	11,40(4,66−27,05)	7,87(4,79−11,23)	**0,021**
IL-6 (pg/mL)	Before	3,48(2,20−5,47)	4,11(2,06−5,43)	0,995
	After	6,09(4,37−9,13)	4,40(2,72−5,32)	**0,002**
Leptin (ng/mL)	Before	6,67(4,38−9,83)	2,36(1,76−3,58)	**<0,001**
	After	5,71(3,76−9,26)	2,38(2,08−3,59)	**<0,001**
VEGF-C (ng/L)	Before	3667,45(2721,59-4872,39)	5357,65(4098,66-6183,00)	**<0,001**
	After	3913,30(3293,74-5150,34)	3580,27(3066,46-4304,51)	0,224

Data are presented as mean±SD or median (IQR) and indicate change from baseline. CG: control group; DG: dietary group

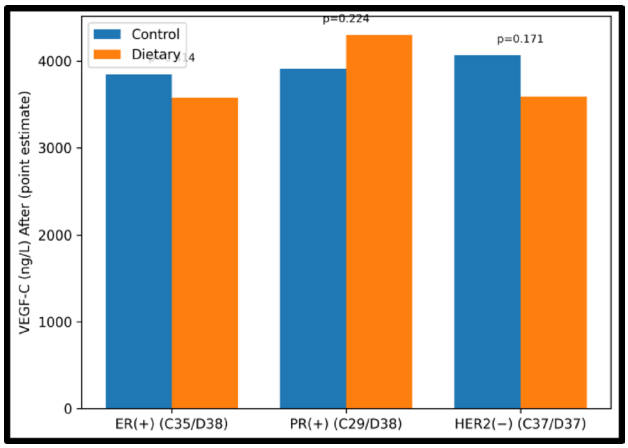


Fig. 1 Post-intervention VEGF-C by subgroups

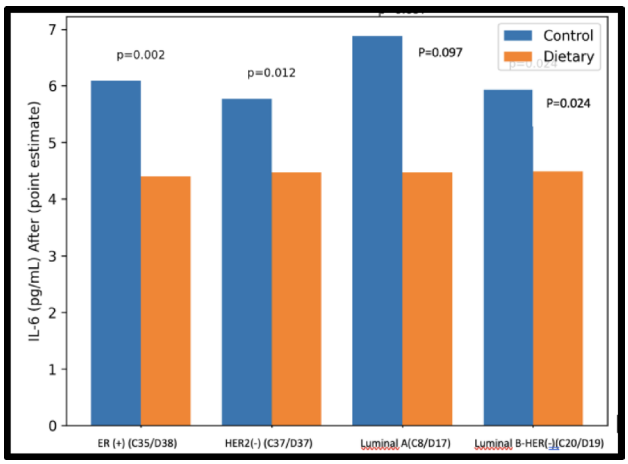


Fig. 2 Post-intervention IL-6 by subgroups

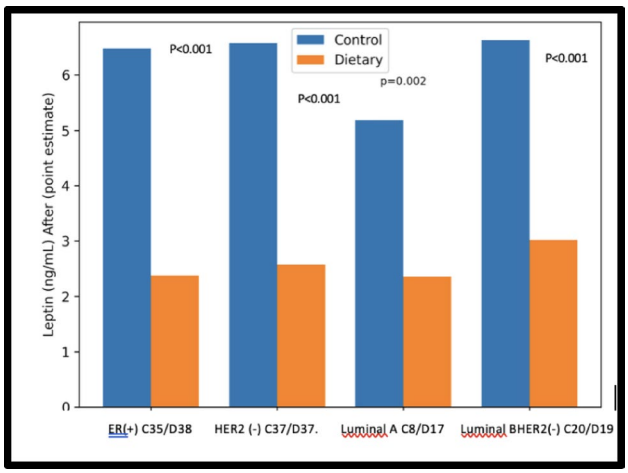


Fig. 3 Post-intervention leptin by subgroup

levels were significantly higher in the dietary group compared to the control group ($p<0.001$) (Table 4) (Fig. 1).

When we compared the data of ER-positive patients between pre- and post-dietary levels, BMI significantly

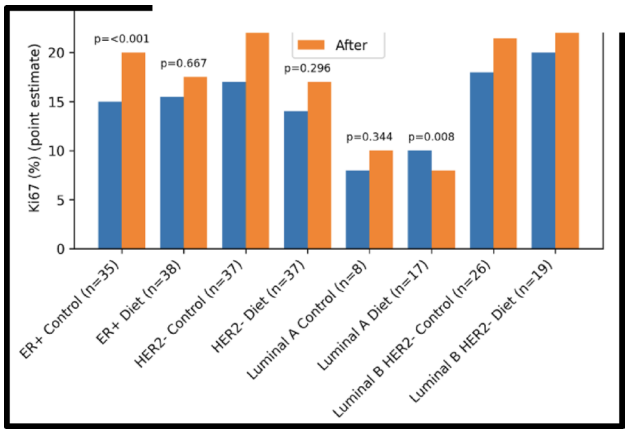


Fig. 4 Comparison of Ki-67 in controls vs. dietary group at time point before vs. after intervention for selected groups

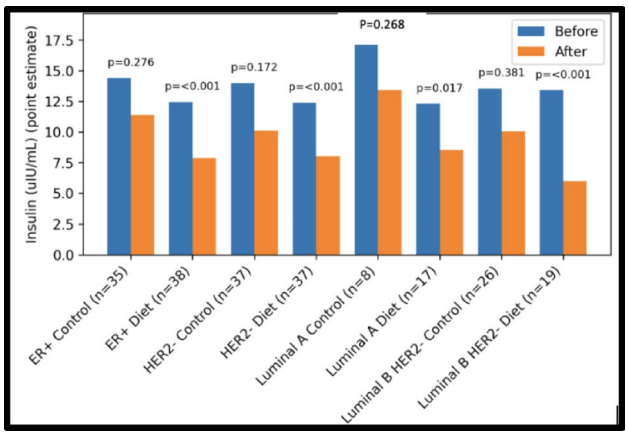


Fig. 5 Insulin levels at diagnosis (before) and immediately after (after) intervention in selected groups

decreased in the dietary group ($p<0.001$). The Ki-67 value increased in both intervention and control groups, but it was only significantly different in the control group. The levels of total cholesterol, HDL cholesterol, LDL cholesterol, albumin, total protein, and IGF-1 significantly decreased during the pre-operative period in both groups (Figs. 6 and 7). In the dietary group, there was a notable decrease in C-peptide, TNF-alpha, and VEGF levels, while IL-6 levels saw a significant rise in the control group. (Table 4) (Fig. 8). When comparing both groups, insulin and C-peptide levels were significantly lower on the day of surgery in the dietary group, whereas IL-6 levels were significantly higher in the control group on the same day. Conversely, VEGF levels were significantly lower in the dietary group after dietary intervention. (Table 4).

For the PR-positive patients, total cholesterol, total cholesterol, HDL cholesterol, LDL cholesterol, albumin, and total protein levels significantly decreased in both groups during the pre-operative period. Moreover, Ki-67 and IL-6 levels was significantly high in the control group, whereas

Table 4 Evaluation of laboratory parameters for Luminal A and Luminal B (HER2 (-) patients in control and dietary groups

Biochemical parameters					
			Luminal A CG ($n=8$)	**Luminal A DG** ($n=17$)	***p***
C-peptide (ng/mL)		Before	3,62(2,35−5,42)	3,32(2,45−4,01)	0,628
		After	3,76(2,24−4,24)	2,34(1,80−2,78)	**0,019**
IL-6 (pg/mL)		Before	3,37(2,51−7,43)	3,70(2,00–5,46)	0,374
		After	6,88(4,58−14,58)	4,47(3,09−5,73)	0,097
Leptin (ng/mL)		Before	6,32(4,95−10,63)	2,44(1,72−3,61)	**<0,001**
		After	5,18(3,71−11,98)	2,36(2,07−3,37)	**0,002**
TNFα (ng/L)		Before	327,14(288,12–474,37)	317,13(309,00-372,09)	1,000
		After	305,70(266,54–558,00)	303,10(275,23–337,81)	0,588
FGF (pg/mL)		Before	956,47(673,25-1040,63)	686,16(480,74–893,68)	0,057
		After	636,79(554,89–855,78)	592,00(515,61–642,29)	0,262
VEGF-C (ng/L)		Before	3298,13(2724,07-3893,83)	4645,31(3922,43-5923,37)	**0,005**
		After	4266,47(3016,08-5277,48)	3330,47(2981,42-4122,11)	0,238
			Luminal B HER2 (-) CG ($n=20$)	**Luminal B HER2 (-) DG** ($n=19$)	**p**
IL-6 (pg/mL)	Before		3,84(2,07−5,12)	4,15(2,28−5,86)	0,628
	After		5,93(4,14−8,81)	4,49(3,14−5,18)	**0,024**
Leptin (ng/mL)	Before		7,81(3,75−10,59)	2,27(1,75−3,65)	**<0,001**
	After		6,63(3,93−10,52)	3,02(2,12−3,75)	**0,001**
VEGF-C (ng/L)	Before		3336,51(2607,14-5042,08)	5749,05(4405,88-6299,72)	**<0,001**
	After		3959,18(3137,33-5135,41)	3840,04(3319,99-4387,60)	0,713

Data are presented as mean ± SD or median (IQR) and indicates change from baseline. CG: control group; DG: dietary group

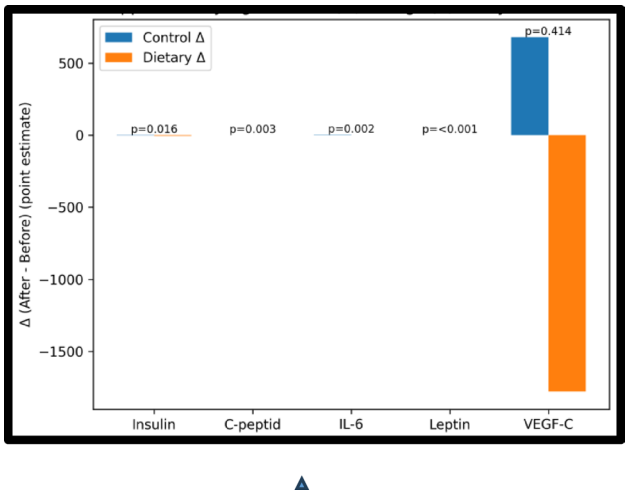


Fig. 6 Changes in lipid profiles and systemic protein/albumin levels in ER(+) patients in the control ($n=35$) and dietary intervention ($n=38$) groups

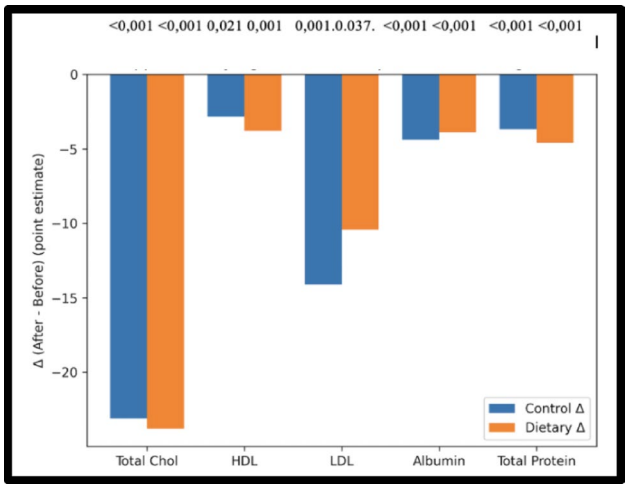


Fig. 7 Change in TNF-alpha and FGF in ER (+) patients in the control ($n=35$) and dietary intervention ($n=38$) groups

bFGF levels was low in the control group. In contrast, BMI, insulin, C-peptide, TNF-alpha, IGF-1, and VEGF levels significantly decreased in the study group (Tables 2, 4 and 5). Furthermore, insulin, C-peptide, leptin, and IL-6 levels were significantly lower on the day of surgery in both groups. At the beginning of the study, leptin levels were lower, whereas VEGF levels were higher in the dietary group (Table 4).

When we evaluated Luminal A group patients, we found that BMI, Ki-67, total cholesterol, insulin, C-peptide, and VEGF levels significantly decreased in the diet group during the pre-operative delay of around 14 days. In both groups, albumin and total protein levels also significantly decreased (Tables 2, 4 and 5). C-peptide and leptin levels were significantly low on the operation day for the dietary intervention group. However, the VEGF level was high at the time of diagnosis in the intervention group (Table 5) (Figs. 6 and 7).

In Luminal B HER2 (-) patients, BMI, insulin, C-peptide, IGF-1, and VEGF levels decreased following dietary intervention in the dietary group. In the control group, Ki-67 and IL-6 levels significantly increased, whereas SHBG, TNF-alpha, and bFGF levels decreased. Total cholesterol, HDL cholesterol, albumin, and total protein levels declined in both groups (Tables 2, 4 and 5). At the beginning of the

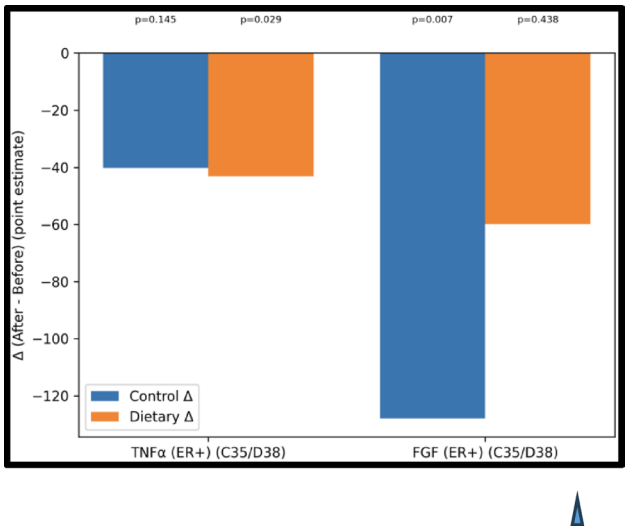


Fig. 8 Change in TNF-alpha and FGF in ER (+) patients in the control (n=35) and dietary intervention (n=38) groups

study, leptin levels were lower, whereas VEGF levels were higher in the dietary intervention group (Table 4). Absolute changes and between-group differences for key outcomes are summarized in Table 6 to facilitate interpretation of effect size.

6 Discussion

Breast cancer is the most prevalent malignancy among women [12]. Notably, a Western-style diet rich in high-caloric animal protein has been hypothesized to contribute to an increased incidence of breast cancer [4]. Many studies have shown that restricting protein in the diet, without reducing calories, is an effective and reliable way to prevent diseases and promote a healthy lifestyle [13, 14]. Especially in cancer patients, dietary protein restriction has been shown to have a negative correlation with cancer incidence, tumor growth, and mortality [15, 16].

Table 6 Absolute Changes (Δ) in Key Outcomes Between Baseline and Surgery

Parameters	CG Δ (Pre→Post)	DG Δ (Pre→Post)	Between groups Δ	*p*
Ki-67 (%)	+5.0 (approx)	+3.0 (approx)	-2.0	0.018
Insulin (uIU/mL)	-3.0	-6.5	-3.5	0.016
C-peptide (ng/mL)	-1.0	-1.8	-0.8	0.038
IL-6 (pg/mL)	+2.3	+0.4	-1.9	0.012
VEGF-C (ng/L)	+565	-1816	-2381	ns

This randomized, open-label preoperative study investigated whether a short-term isocaloric dietary intervention differing only in protein composition, combined with a moderate increase in physical activity for low-protein diet patients, may influence tumor biology and systemic metabolic-inflammatory markers during the short interval between diagnosis and surgery in early-stage breast cancer. The principal finding was that, within a surprisingly short preoperative window of around 13–14 days, measurable changes occurred in BMI and circulating metabolic and inflammatory biomarkers and, to a limited extent, in tumor proliferation indices, which were restricted to the widely used Ki-67 index in this study. However, these effects were heterogeneous across subgroups and should be interpreted cautiously in light of methodological constraints [12].

We observed a significant decrease in the mean BMI of the low-protein intervention group, while the mean BMI of the control group did not change. These results are consistent with a previous study by Li Sun et al., which showed a positive correlation between tumor size in breast cancer

Table 5 Biochemical changes during dietary intervention for ER(+), PR(+), HER2 (-) patients in both groups

		Ki67(%)		IGF-1(ug/L)		IL-6 (pg/mL)		FGF (pg/mL)		VEGFc (ng/L)		TNFα (ng/L)	
		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
ER(+)	CG (n=35)	15,00(10,00-20,00)	20,00(16,00-28,00)	132,64(+/-43,37)	124,22(+/-36,27)	3,68(2,24-5,19)	6,09(4,23-9,63)	716,36(617,42-957,78)	588,36(509,73-845,36)	3169,97(2711,13-4570,41)	3850,21(3018,73-5120,48)	333,85(282,20-491,02)	293,65(252,90-445,12)
	DG (n=38)	15,50(9,75-25,00)	17,50(8,00-26,00)	131,98(+/-42,69)	119,63(+/-37,61)	4,11(2,06-5,42)	4,39(2,71-5,32)	677,52(482,13-950,15)	617,68(526,06-791,39)	5357,64(4098,65-6182,99)	3580,26(3066,46-4304,50)	354,35(308,78-393,36)	311,17(280,75-338,03)
PR(+)	CG (n=29)	17,00(10,00-20,50)	22,00(16,50-29,00)	134,44(+/-43,94)	128,44(+/-40,55)	3,48(2,19-5,47)	6,09(4,37-9,13)	705,89(614,71-968,68)	564,84(505,42-828,05)	3667,44(2721,59-4872,38)	3913,30(3293,73-5150,33)	328,12(283,00-515,37)	278,07(246,32-365,61)
	DG (n=38)	15,50(9,75-25,00)	17,50(8,00-26,00)	131,98(+/-42,69)	119,63(+/-37,61)	4,11(2,06-5,42)	4,39(2,71-5,32)	677,52(482,13-950,15)	617,68(526,06-791,39)	5357,64(4098,65-6182,99)	3580,26(3066,46-4304,50)	354,35(308,78-393,36)	311,17(280,75-338,03)
HER2 (-)	CG (n=37)	17,00(10,00-24,00)	131,50(+/42,91)	3,48(2,12-5,47)	5,77(4,12-9,00)	126,74(+/-39,73)	22,00(16,50-32,00)	695,36(609,63-954,63)	686,15(480,73-955,89)	3503,05(2662,17-4641,16)	5408,27(4060,41-6195,87)		
	DG (n=37)	14,00 (9,50-25,00)	131,39(+/44,70)	4,07(2,04-5,55)	4,47(3,10-5,32)	118,41(+/-38,25)	17,00 (8,00-25,50)	588,36(512,57-837,92)	638,84(530,60-793,78)	4068,14(3128,56-5193,27)	3591,99(3093,34-4223,07)		

ER(+) CG before/after Ki-67 p=0.001, IGF-1 p=0.033, IL6 p=0.011 FGF p=0.007; DG before/after TNF p=0.029 IGF-1 p<0.05, VEGFc p=0.001
PR(+) CG before/after Ki-67 p=0.001, IL6 p=0.033, FGF p=0.01; DG before/after TNF p=0.029 IGF-1 p=0.014, VEGFc p=0.001
HER2(-) CG before/after Ki-67 p=0.001, IL6 p=0.012, FGF p=0.011; DG before/after IGF-1 p=0.011, VEGFc p=0.001

and high BMI, adversely affecting disease prognosis [17]. Furthermore, Fontana et al. reported a study done on human xenograft models and these authors showed that a 7% protein-restricted isocaloric diet led to a 56% reduction in tumor volume compared to animals receiving the 21% protein diet [9].

An interesting finding of our study was the decrease in selected inflammatory mediators and circulating leptin in the dietary intervention group. These findings are biologically plausible and substantiate the suggestion that short-term metabolic modulation may rapidly affect systemic signaling pathways pertinent to tumor biology. Insulin resistance and hyperinsulinemia are known to activate the IGF-1/PI3K/AKT/mTOR axis, which promotes tumor growth and survival [18–20]. Research has indicated that reduction of isocaloric protein consumption in human xenograft models decreases serum IGF-1 levels and inhibits the PI3K/Akt/mTOR complex and suppresses cell proliferation as implied by reduced Ki-67 levels [9]. IGF-1 has been shown to contribute to breast cancer development by promoting cell proliferation, migration, and angiogenesis through the activation of the PI3K-Akt, mTOR complex, and MAPK signaling pathways [21]. Elevated circulating IGF-1 levels have been implicated in breast cancer pathogenesis [8]. Research has shown that reducing protein intake, while creating a caloric deficit, can lead to lower levels of IGF-1 [15]. The present study found a decrease in IGF-1 levels within the protein-restricted diet group, especially among the HER2-negative, ER-positive, PR-positive, and Luminal B HER2-negative subgroups, that may be associated with these mechanisms. However, there were no significant changes in IGF-1 levels observed after protein restriction in patients within the Luminal A subgroup, possibly because of the small group size. No significant variations in IGF-1 levels were detected in the control groups within the HER2-negative, PR-positive, and Luminal B HER2-negative subgroups.

Ki-67 is a widely used marker of cell proliferation, and Ki-67 elevation is correlated with poorer survival and prognosis and increased local recurrence [22]. Although the systemic changes observed in the present study were beneficial, their effects on tumor proliferation, as reflected by Ki-67, were modest and inconsistent across subgroups. While a decrease in Ki-67 was detected in certain groups, the magnitude of change was small and did not consistently occur across all intrinsic tumor subtypes. This finding highlights the intricacy of tumor biology and indicates that short-term metabolic treatments may exert varied effects depending on baseline patient and underlying tumor characteristics. We hypothesize that less proliferative luminal cancers may exhibit less sensitivity to short-term metabolic disturbances, whereas biologically more active tumors may have a greater response. The Ki-67 ratio before and after the diet in the control group, except for the Luminal A molecular subgroup, showed a significant increase in all other subgroups. Although the mean Ki-67 value increased in the luminal A group, it was not significant. In the study group, there was no significant difference in the other subgroups, except for the Luminal A group, where a statistically significant decrease was observed. Interestingly, we found that protein-restricted diet decreased Ki67 in the luminal A group and did not increase it in other subgroups compared to the control group. However, given the very small subgroup sizes, this finding should be considered with caution until tested in much larger studies. In contrast to a previous study which reported that limiting protein intake led to diminished IGF-1 levels and a reduced Ki-67 ratio, the present study found no significant alteration in Ki-67 ratios among participants adhering to a protein-restricted diet. However, due to the restricted sample size and uneven subtype distribution, these observations must remain hypothesis-generating rather than definitive [17, 21, 22].

The heterogeneity of response observed in the present study aligns with emerging evidence that metabolic interventions may interact with tumor-intrinsic biology in a subtype-dependent manner. Breast cancer is understood as a group of biologically diverse diseases, and therapies targeting systemic metabolism may not produce consistent effects across these subtypes. In this context, our findings support the imperative of stratifying future studies according to intrinsic molecular subtype rather than exclusively depending on receptor-based classifications [23–25].

Another interesting result is the reduction in leptin levels and its potential correlation with angiogenic signaling pathways. Leptin has been implicated in tumor angiogenesis interactions with VEGF signaling. Although there were changes in VEGF-C, they were not statistically robust, suggesting that the brief duration of intervention may be too short to induce consistent changes in angiogenic pathways or that the baseline differences in this biomarker may have skewed our findings. Similarly, the lack of substantial alterations in certain growth factors, such as bFGF, indicated that structural remodeling processes may need longer intervention durations [26–29].

Importantly, this study included a relatively short preoperative interval of about two weeks, which may reasonably be expected to limit the magnitude of biological change that may occur. Tumor growth kinetics and microenvironmental remodeling are dynamic but may not respond within such a constrained timeframe, particularly because significant biological changes typically require longer durations to manifest fully. Therefore, the modest changes observed in tumor proliferation markers should not be interpreted as evidence of clinical tumor control, but rather as preliminary signals

that short-term metabolic interventions may influence biologically relevant pathways [30, 31].

Several methodological limitations must be considered when interpreting these findings. First, the study was not registered in advance, which makes transparency difficult and leads to potential reporting bias. Second, even though randomization was performed, baseline imbalance in intrinsic molecular subtypes represents an important source of confounding, particularly for outcomes such as Ki-67 and tumor size changes. Third, no multivariable adjusted analyses were performed due to limited sample size and risk of model overfitting. Therefore, potential confounding due to baseline imbalance in intrinsic molecular subtypes cannot be excluded. We must emphasize that findings should be interpreted as exploratory. Exploratory adjusted analyses were considered; however, given the modest subgroup sizes, results were deemed statistically unreliable and therefore not reported. The sample size was modest, and subgroup analyses would have been underpowered, increasing the risk of both Type I and Type II errors. Fourth, many comparisons were made without formal adjustment, and therefore statistically significant results should be interpreted with caution. Fifth, although physical activity was objectively monitored, dietary adherence was monitored through repeated assessments but was not objectively validated using biochemical markers. This introduces potential exposure misclassification, which may attenuate observed associations and affect internal validity [32–35].

In addition, the open-label approach also makes it possible for performance bias to occur, although outcome evaluation was performed in a blinded way. Finally, the lack of long-term oncologic outcomes precludes any inferences regarding recurrence, progression, or survival. These limitations collectively confirm that the present study results should be viewed as exploratory [36, 37].

From a clinical perspective, the findings do not support the use of short-term dietary intervention as a strategy for tumor control or as a substitute for timely surgical intervention. Instead, they suggest that even a short preoperative window may present a biologically useful period during which systemic metabolic modulation may affect tumor-related pathways. This idea aligns with the growing interest in window-of-opportunity trials, which investigate biological effects of interventions over short durations without delaying regular care.

Subsequent studies should be designed with prospective registration, established outcomes, and adequate statistical power. Furthermore, they should integrate stratification by intrinsic molecular subtype. Longer intervention durations, incorporation of molecular profiling, and assessment of downstream signaling cascades may elucidate the relationship between metabolic therapies and changes in tumor biology. Furthermore, the inclusion of clinically significant endpoints will be crucial to determine if these biologic changes translate into therapeutic benefit.

7 Conclusion

Short-term lifestyle and dietary changes during the two-week preoperative phase influenced systemic metabolic and inflammatory markers and were associated with modest changes in tumor growth and proliferation biomarkers in selected subgroups. However, owing to limited sample size, the exploratory design, subtype imbalance,, and absence of multiplicity adjustment, these results should be interpreted with caution. Before any treatment can be contemplated, prospectively registered, larger and subtype-stratified studies with clinically significant objectives are necessary.

Author contributions EK contributed to the concept, design, and implementation of the study, conducted the plausibility testing, and drafted the report. NZC was involved in the study design and assisted in drafting the report. TŞ, SAG and ÖDÖ participated in the concept, design, and implementation of the study, as well as the drafting of the report. All authors confirm their contributions as stated and have reviewed and approved the final version of the manuscript.

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Data Availability All data was generated with laboratory work from blood samples of patients in both groups by authors. Informed consent was taken from each patient.

Declarations

Conflict of interest None of the authors had any conflicts of interest. This work was supported by Kocaeli University Research Fund with BAP Code:2022–2822.

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