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Effects of Ketogenic Metabolic Therapy on Patients with Breast Cancer: A Randomized Controlled Clinical Trial

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Running title: Ketogenic Metabolic Therapy in breast cancer treatment

Data described in the manuscript, code book, and analytic code will be made available upon request pending

Abstract

Background: Ketogenic metabolic therapy (KMT) using ketogenic diets (KD) is emerging as viable alternative or complementary strategy for managing cancer; however, few clinical trials have been reported. The present study aimed to evaluate the effects of a KD in patients with locally advanced and metastatic breast cancer receiving chemotherapy.

Methods: A total of 80 patients undergoing treatment with chemotherapy were randomly assigned to KD or control group for 12 weeks. Concurrent with the admission, midway point , and at 12 weeks, fasting blood samples were collected for evaluation of insulin, IGF-1, CEA, CA15-3, ESR, CRP, IL-10, and TNF-α. Sonography for patients with locally advanced disease and CT or MRI scans for patients with metastatic disease were done on admission and at 12 weeks. At the completion of the chemotherapy, patients with locally advanced disease underwent surgery and stage was recalculated. Also patients with metastases were evaluated for response rate.

Results: TNF-α decreased significantly after 12 weeks of treatment (MD: 0.64 [CI 95%: -3.7, 5] P<0.001), while IL-10 increased (MD: 0.95 [CI 95%: -1,3] P < 0.001) in the intervention compared to the control group. Patients in the KD group had lower adjusted serum insulin compared to the control group (MD:-1.1 [CI 95%: -3,1] p < 0.002). KD lead to a reduction in tumor size in the KD compared to the control (27 vs 6 mm, P=0.01). Stage decreased significantly in patients with locally advanced disease in the KD group after 12 weeks (P < 0.01). No significant differences in response rate were observed in patients with metastatic disease.

Conclusions: KMT in breast cancer patients might exert beneficial effects through decreasing TNF-α and insulin and increasing IL-10. KD may result in a better response through reductions in tumor size and downstaging in patients with locally advanced disease; however, more studies are needed to elucidate the potential beneficial effects of KD in patients with metastases.

Keywords: Ketogenic diet, ketogenic metabolic therapy, breast cancer, response rate, growth factors, anti-inflammatory factor, tumor size

Introduction

Ketogenic metabolic therapy (KMT) is emerging as a novel complementary or alternative therapeutic strategy for a broad range of malignant cancers including breast cancer [1-12]. Calorie restriction and low-carbohydrate high-fat ketogenic diets (KD) reduce the glucose needed to drive the Warburg effect while also elevating ketone bodies [13, 14]. Hypoxia has significant effects on pathogenesis, migration, and metastasis in breast cancer. Hypoxia is associated with the upregulation of glycolysis that can increase acidification in the tumor microenvironment. Hypoxia-inducible factor 1-α (HIF-α) is associated with aggressive growth, metastasis, and poor response to treatment [15]. Of interest, mean HIF-α expression increases from 0% in normal breast tissue to 14.9% in DCIS and to 15.7% in invasive breast cancer [15]. With implementation of a KD, HIF-α decreased [16].

Cancer cells cannot effectively use ketone bodies or fatty acids for ATP synthesis through oxidative phosphorylation due to defects in the number, structure, and function of their mitochondria [13, 17]. Moreover, ketone bodies and fatty acids cannot be fermented, and thus cannot effectively replace glucose as an alternative energy source for cancer [14, 17]. Ketone bodies inhibit glycolysis which in turn decreases the main energy production pathway for cancer cells [18-20]. Bartmann et al. showed that beta-hydroxybutyrate (βHB), the most prevalent ketone body, could not stimulate breast tumor growth in vitro [21]. KD reduces activity in insulin-like growth factor-1 (IGF-1)/insulin–PI3K–Akt–mTOR signaling pathways that have been shown to be correlated with significant tumor growth [22]. KD reduces the peritumoral inflammation and edema that facilitates the growth and metastasis of cancer cells [23]. Hence, KMT becomes a putative therapeutic strategy for managing most cancers including breast cancer [13, 14].

The objective of this study was to examine the effects of 12 weeks of KD treatment on response rate, tumor markers, inflammatory/anti-inflammatory markers, and growth factors in patients with locally advanced and metastatic breast cancer. The treatment protocol of this trial [24] and results related to body composition, feasibility, safety, glucose, blood βHB, liver and kidney markers, have been previously published [8].

Methods

Participants: The study was conducted at the medical oncology clinic, Shohada-e Tarjirish hospital, Cancer Research Center, Tehran, Iran from July 2017 to October 2018. All locally advanced and metastatic breast cancer patients between the ages of 18 and 70 with a biopsy-proven malignancy and undergoing chemotherapy for at least 12 weeks were evaluated for inclusion. The study did not include patients with significant cardiac, renal or neurologic comorbidities, or with malnutrition, diabetes, pregnancy, or a Karnofsky index less than 70. All participants provided written informed consent prior to the study.

This study was a randomized clinical trial with parallel arm design. The study protocol was approved by the National Nutrition and Food Technology Research Institute (NNFTRI), Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran (IR.SBMU.NNFTRI.REC.1396.187). Patients were randomized using block balanced randomization method in a 1:1 ratio into the intervention (n=40) and control (n=40) groups. Block size was 6. This protocol was computer-generated by a statistician who was not a member of the patient's medical team. Due to the nature of diet intervention study, blinding the participants or study personnel was not feasible. The project coordinator enrolled and assigned participants to their interventions.

A eucaloric medium-chain triglyceride (MCT) based KD (comprised of 6% calories from CHO, 19% from PRO, 20% from MCT, and 55% from FAT) was assigned to the patients in the intervention group for 90 consecutive days concurrent with the first 12 weeks of chemotherapy. The calorie requirements and menu were determined for each patient in consultation with a nutritionist, prior to the study. Calorie needs were calculated according to the Mifflin-St. Jeor formula. Other methods were as we described previously [8]. The dietary recommendations were further individualized to enhance patient compliance. Patients were monitored for compliance and possible adverse effects and allowed to contact the nutritionist whenever needed. USDA Standard Reference Database was used to calculate the nutrient composition of the diet. Each patient was provided with MCT oil (500 ml) from Nutricia (Erlangen, Germany) every two weeks. To reduce the risk of adverse gastrointestinal effects, the MCT dosage was gradually increased during the first 6 days until reaching the maximum dose (average of 48 ml daily) then tapered down after 12 weeks. KD administration was also initiated and terminated in a stepwise manner. The patients in the control group followed a standard diet containing 55% CHO, 15% protein, and 30% fat. Diet compliance was verified through assessment of blood βHB levels and dietary intake. Therapeutic ketosis was defined as serum βHB concentrations > 0.3 mmol/l, as we described previously [25].

Response rate was the primary endpoint of this study. Assessment of secondary endpoints included insulin, IGF-1, TNF-α, IL-10, carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3), ESR and CRP.

Clinical and biochemical measurements

Fasting blood sampling for serum insulin, IGF-1, TNF-α, IL-10, CEA, and CA15-3 was performed on admission, at the midway point or 1st follow-up or 6-week, and at the end (12

weeks) of the study. Imaging at baseline and end of the study was performed by a radiologist and included sonography for locally advanced disease, CT scans for metastases to liver and lung and MRI for metastases to bone. Restaging was performed in locally advanced patients that underwent surgery following chemotherapy. IGF-1 and insulin were measured by ELISA (Abcam, USA). (Intra-assay CV of <12%). TNF-α and IL-10 were also measured by ELISA (Aviscera bioscience, USA) (Intra-assay CV of 10-12% for TNF-α and 8-10% for IL-10). Chemiluminescence was used for quantification of CEA and CA15-3. CRP was measured using a Photometry Method via (Roche Hitachi 912, Basel, Sweden). ESR was evaluated manually. Status of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), ki67, and tumor stage were obtained from patient records. Perineural invasion (PNI), lymphovascular invasion (LVI), stage calculate accord TNM index (Tumor, Node, Metastasis), and lymph node score were obtained from postoperative pathology reports. Response rate was determined by evaluation of changes in tumor size. Sonography as well as postoperative pathology reports was used to evaluate tumor size in locally advanced patients.

Statistical methods

Sample size was calculated to detect a 30% difference in response rate (tumor size) between the two groups. Considering the 80% power and an alpha level of 0.05, the sample size was calculated as 30 individuals per group. Assuming a 20% dropout rate during 12 weeks of the study, the final number of participants needed was determined to be 40 patients in each group.

Statistical analysis was carried out according to the intention-to-treat protocol. Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test then reported as mean ± standard deviation or median as appropriate. Student t-test or Mann–Whitney U test was used to compare continuous variables between groups. Categorical data were summarized as

153 percentages and analyzed through Chi-square or Fisher tests. ANOVA was used to evaluate the
154 differences in the baseline, midpoint, and endpoints in time-dependent variables within patient
155 groups. The ANCOVA test was used to eliminate the effect of confounding factors.
156 Data were analyzed using the SPSS version 18.0 software (Chicago, IL, USA) and stata version
157 13. $P<0.05$ was considered as statistically significant. Bonferroni correction was applied for
158 multiple comparisons if appropriate.

159 **Results**

160 Initially, a total of 80 women with breast cancer were randomly assigned to the KD group (n =
161 40) and the control group (n = 40). Thirty patients in each group completed the study (Fig 1).
162 Baseline characteristics of the participants are listed in Table 1**.** There were no significant
163 differences between groups with regard to age, cancer type, metastasis vs locally advanced
164 disease, or ER, PR, HER2 status ($P > 0.05$). Three-day average nutrient intake at baseline and at
165 12 weeks are shown in Table 2. There was no significant difference between groups in baseline
166 variables (protein, carbohydrate, fat and total energy, $P > 0.05$). Despite we did not prescribe a
167 calorie restricted ketogenic diet, KD group showed a significant reduction in calorie intake
168 compared to the control group $P < 0.01$.
169 In our study, 75% of the patients in the KD group completed the trial. Based on the standard of
170 βHB > 0.3 mmol, 89% of all participants who completed the KD, were considered compliant to
171 the diet.
172 Table 3 presents data reflecting patient outcomes at different time intervals for each trial arm of
173 the study. TNF-α levels (μmol/ml) remained constant over the course of the study in the control
174 group (17.6 ± 8.6 to 17.3 ± 7.3, $P = 0.999$) but decreased from 21.9 ± 8.8 to 18 ± 8.6 in the KD
175 group ($P < 0.001$). The difference between groups was significant at the end of the study ($P <$

176 0.001). During the follow-up period, plasma levels of IL-10 (ng/ml) increased significantly in the
177 KD group (from 9.1 ± 4.4 to 11.1 ±4 .7, P < 0.001), but remained unchanged in the control group
178 (10.4 ± 4.5 to 10.1 ± 4.3, P = 0.999). IL-10 levels were significantly higher in KD group than in
179 the control group at the end of the study (P < 0.001). No significant differences were seen for
180 ESR or CRP either within or between groups.
181 A significant difference was seen for insulin levels between the two groups (P < 0.002) after
182 adjusting for baseline insulin, weight loss, and dissimilarities in caloric intake. However, no
183 significant difference was found between groups for IGF-1 (P = 0.77) (Table 4). After adjusting
184 for stage and cancer type (metastatic compared to locally advanced), significance remained.
185 A significant decrease in insulin and IGF-1 levels was also observed in the intervention group at
186 the end of the study compared to the baseline (P = 0.03 and P = 0.02, respectively) (Table 4).
187 The effect (regression coefficient) of time on the outcome variables such as insulin, TNF-α and
188 IL-10 was significant (P = 0.01 and P = 0,004, P = 0.01, respectively) .
189 Changes in tumor markers during the study period are shown in Figure 2. No significant changes
190 were observed in CEA and CA 15-3 either within or between groups.
191 Data regarding the effects of KD on response rate in patients with locally advanced disease are
192 shown in Table 5. Based on both sonography and pathology reports, at the end of the study
193 reduction in tumor size was 27 mm in the intervention group compared to 6 mm in the control
194 group (P < 0.01).
195 Post-surgery PNI and LVI showed no significant differences between the two groups. However,
196 there was a significant decrease in TNM index in the KD group compared to the control group at
197 the endpoint (P < 0.01) (Table 6). The response rate in metastatic patients showed no significant
198 difference between the two groups (p = 0.48). In KD group, 3 patients (bone, lung, lung with

bone) had progression disease and 2 (liver, lung with bone) achieved a partial response. In control group, there were 2 (liver, bone with liver) partial responses, 4 (3 bone, 1 long) stable disease and 2 (bone) progression. (Data not shown)

Discussion

This study evaluated the effects KMT on tumor markers, inflammatory/anti-inflammatory markers, and growth factors as well as response rates in patients with locally advanced and metastatic breast cancer at a single institution. We found that IL-10 was higher while TNF-α, insulin, IGF-1, tumor size, and TNM were lower in the KD group than in the control group. No significant differences were found between the groups in ESR, CRP, CEA, and CA 15-3.

Effect of diet on inflammatory and anti-inflammatory factors

To the best of our knowledge, no prior study has evaluated the effects of KMT on TNF-α and IL-10 in cancer patients. In the Paoli study of overweight males, TNF-α showed a significant decrease in KD subjects; however, no significant changes were seen for the anti-inflammatory cytokine IL-10. Consistent with our findings, Klement et al. also found no significant changes in the CRP levels in 6 cancer patients [26].

Low IL-10 expression is associated with recurrence, metastasis, and poor survival in breast cancer patients [27]. It is widely accepted that IL-10 exhibits an anti-tumorigenic effect by downregulating the synthesis of VEGF, IL-1b, TNF-α, IL-6, and MMP-9 needed to sustain the enhanced angiogenesis that accompanies tumor progression.

Peritumoral inflammation, arising largely from lactate accumulation in the tumor microenvironment, is a condition favoring the growth and metastasis of cancer cells [14, 28]. TNF-α increases the growth and metastasis of breast cancer cells through stimulating the

expression of miRNA-23b and miRNA-27b, MMP-9 and inhibition of Nischarin [29]. KD results in the suppression of TNF-α expression via PPARγ activation [30].

Effect of diet on growth factors

We found that fasting insulin levels were lower in the KD group than in the control group. The trend for IGF-I in the KD group showed a significant decrease compared to baseline; however, this trend was not observed in the control group. Consistent with our findings, Cohen and colleagues found that fasting insulin was lower in the KD group than in the control group, but levels of IGF-1 were not significantly different between the two groups [31]. In contrast with our results, Klement found no significant difference between baseline and end of study for insulin and IGF-1levels [26].

Higher levels of insulin and IGF-1 may predict a higher risk of recurrence and mortality in breast cancer survivors [32-34]. They exert their effects through PI3K/Akt, Ras/MAPK, and b-catenin signaling pathways [35-37]. Previously, we showed that KD caused a 20 mg/dl decrease in fasting blood glucose (FBG) and increased βHB levels in breast cancer patients [8]. Thus, KD leads to positive clinical effects by lowering blood glucose levels with subsequent insulin reduction in patients with breast cancer.

Effect of diet on tumor markers

After the intervention period, no significant differences were seen in CEA and CA 15-3 between the two groups, confirming the results of a previous report by Freedland on the effects of low-carbohydrate diet plus walking in prostate cancer patients. In that study, no differences were found in PSA at 3 and 6 month follow-up between groups [38]. In Yang's study, elevated levels of CA15-3 were reported to be associated with a poorer prognosis and increased risk of metastasis [39].

Effects of KD on response rate

KD led to a significant decrease in stage and tumor size compared to the control group. The tumor size in KD group showed a significant reduction compared to the baseline; reduction in tumor size was 27 mm in the intervention group compared to 6 mm the control group. Also, lymph node scores (N1, N2, N3) decreased from baseline to the end of the study in the KD group. This trend was not seen in the control group.

KDs provide an inhospitable microenvironment for cancer cell growth [40]. The reductions in tumor size and TNM index may be due to the effect of KD on insulin, FBG, IL-10, TNF-α, oxidative stress and other factors. In our study, FBG and lactate were decreased thus reducing glycolysis; as a result, response to treatment may have been enhanced.

To date, a number of studies investigating KDs and cancer have been conducted in metastatic patients, in our study, no significant differences in response rate were observed between the intervention and control groups. In five patients in the KD group, one had stable disease, two had partial response, and three patients showed disease progression. Due to the low sample size and heterogeneity in previous studies, a statistical evaluation of the diet effect on tumor characteristics was not feasible. Moreover, the response rate was higher in patients at a lower stage and in patients with stable disease compared to patients with more advanced disease [41]. While some patients had a favorable response to treatment, others showed disease progression. Due to the heterogeneity of patients in these studies, it is not clear which patients may benefit the most from KD therapy.

In Schmidt's study of the effects of a ketogenic diet in patients with advanced cancer, the disease progressed in five patients who then discontinued the diet, whereas five patients who adhered to the diet throughout the study had stable disease [41]. In another study, chemotherapy combined

with KD resulted in tumor regression in five early-stage patients; however, this outcome was not seen for patients with metastatic small cell lung cancer [26]. In another trial, progressive disease was seen in four patients and stable disease in five [42].

The difference in the results seen in these studies may be attributed in part to study design, cancer type, disease duration, and even to each individuals' unique metabolic status. Still, according to our findings, it appears that the application of KD diet therapy in locally advanced patients may be of greater benefit than in metastatic patients. A recent systematic review has proposed that more high-quality controlled trials are needed to develop the evidence for the use of KD in clinical practice [43]. Contradictory results have been reported regarding the effect of βHB on cultured tumor cells, suggesting that the effect on cancer cell growth was dependent on tumor energetic conditions that support the utilization of βHB as an energy source for oxidative cells, resulting in faster growth of tumors with predominantly oxidative cells [44]. It is not clear, however, how breast cancer cells with defective mitochondria could obtain energy from ketone bodies [14, 45]. Moreover, if tumor cells could use fatty acids and βHB for growth, then water-only fasting and calorie-restricted KD should accelerate tumor growth [46, 47]. This was clearly not the case for our patients. According to Bartman there is no association between βHB or acetoacetate and breast cancer cell proliferation or response to treatment [48]. Previous studies showed that ketone bodies could inhibit growth of glioma and melanoma cells [19, 49]. In our study, elevated βHB levels were linked to reduced tumor growth.

Previously we reported the overall survival rate among locally advanced patients was higher in KD compared to the control group [8]. This may have been due in part to the increase in IL-10, decrease in insulin, decrease in TNF- α, or decrease in the stage of the cancer in the KD group.

Despite a nearly century-long history of use, there are still ongoing concerns about the side effects of KDs. These are widely understood and rarely lead to discontinuation of the diet; they should be routinely monitored during clinic visits that include lab testing of serum chemistries, blood counts, fasting lipids, and kidney/liver function. Some effects are predictable and preventable; others, such as dehydration and electrolyte imbalances, are easily treatable. We previously reported that no serious complications or adverse effects were observed in a KD intervention group [8].

The present study has several strengths. To the best of our knowledge, this is the first randomized controlled trial examining the effects of KD on breast cancer patient biomarkers and tumor size. However, variations in stage and grade of the cancers in this population of patients was a limitation of our study. Future studies should also address ways to better control for variations between individuals in MCT intake as tolerance issues may account for differences we observed in βHB levels. Although this study is one of the largest of its type conducted to date, its small sample size only allowed for detection of large effects. Hence larger trials that address these limitations are needed.

Conclusions:

We conclude that application of KMT for 12 weeks can have beneficial effects in breast cancer patients through inhibitory effects on inflammatory biomarkers and growth factors, and through enhancement of the anti-inflammatory factor, IL-10. Our findings show that a KD results in a reduction in tumor size and stage in locally advanced breast cancer patients, possibly by creating a metabolic environment that inhibits tumor progression. However, more studies are needed to elucidate the potential beneficial effects of KD in patients with metastatic cancer.

Conflict of interest:

The authors declare that they have no competing interests

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Not applicable

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Authorship:

Khodabakhshi carried out the conception, developed the methodology, performed the experiments and wrote the article. Mirzaei, Akbari, Davoodi, Kalamian, and Seyfried collaborated on the design of the study. Akbari and Mirzaei provided access to the patients. Davoodi supervised the dissertation project. Kalamian and Seyfried critically reviewed the manuscript. All authors have read and approved the final manuscript.

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Table 1) Baseline characteristics in breast cancer patients before KMT

	Scale categories	**Intervention (Ketogenic diet) n=30**	**Control (Ordinary) n=30**	**P value**
Age; Mean ± SD	year	44.8 ± 8.4	45.2 ± 15.0	0.91†
Cancer Type	Loc Adv	25 (83.3)	19 (63.3)	0.08$
	Met	5 (16.7)	11 (36.7)	
ER	positive	22 (73.3)	20 (66.7)	0.57$
	negative	8 (26.7)	10 (33.3)	
PR	positive	15 (50)	18 (60)	0.43$
	negative	15 (50)	12 (40)	
HER2	positive	12 (40)	13 (43.3)	0.79$
	negative	18 (60)	17 (56.7)	
Ki67	positive	8 (33.3)	6 (23.1)	0.42$
	negative	16 (66.7)	20 (76.9)	
Stage	2	14 (46.7)	11 (36.7)	0.07$
	3	9 (30)	4 (13.3)	
	4	7 (23.3)	15 (50)	

† calculated by independent t-test

$calculated by chi square test

Categorical data shown as n (%)

KMT: Ketogenic Metabolic Therapy

ER: Estrogen receptor PR: Progesterone receptor

HER2: Human epidermal growth factor receptor 2

Table 2) 3-day average nutrient intake at baseline and 12-weeks in breast cancer patients by two trial arms

Variable	KD		Control	
	Week 0	Week 12	Week 0	Week 12
Protein (g)	72(62-79)	57(47-68)	72(47-88)	69(60-86)
Fat (g)	57(50-61)	107(72-123)**	61(50-72)	55(41-61)
Carbohydrate (g)	245(195-270)	22(14-27)**	250(201-277)	210(147-241)
Energy (Kcal/day)	1704(1530-1870)	1263(937-1516)*	1767(1507-2033)	1600(1482-1750)

Values are median (percentile 25-75) *$P < 0.01$; **$P < 0.001$ compared with week 12 control group
KD: Ketogenic diet

Table 3) Biomarker levels in control and KMT-treated breast cancer patients

Variable	Trial arms	Baseline	Midway point	12 weeks	P value MP vs BL	P value 12 wks vs MP	P value 12 wks vs BL
TNF-α	KD	21.9±8.8	19±9.1	18±8.6	0.001	1	<0.001
(µmol/ml)	Control	17.6±8.6	16.4±6	17.3±7.3	1	0.21	1
Between groups	MD (95% CI)	4.2 (-0.4,9)	2.6 (-2.6,7)	0.64 (-3.7, 5)	0.16	1	<0.001*
IL-10	KD	9.1±4.4	10.6±4.5	11.1±4.7	0.11	0.21	<0.001
(ng/ml)	Control	10.4±4.5	10±4.5	10.1±4.3	1	1	1
Between groups	MD (95% CI)	-1.3 (-3.7,1)	1.5 (-2,3)	0.95 (-1,3)	0.79	1	0.001*
ESR	KD	27±21	32.1±15	35.1±19	0.99	1	0.23
	Control	28.4±16	41.1±23	33.6±15	0.13	1	0.16
Between groups	MD (95% CI)	-1.3 (12.3,9.6)	-5.8 (21.4,4.2)	-1.5 (8.6,11.6)	1	0.47	1
CRP	KD	9±14	11±13	12±13	1	1	1
	Control	10±14	18.6±18	14.3±14	0.16	1	0.19
Between groups	MD (95% CI)	-1 (-9,7)	-7 (-17,3)	-2 (-10,6)	1	0.32	1

BL: Baseline, Midway point: MP 1st follow-up or 6-week, 12 -weeks or last follow-up, MD: Mean Difference, CI: Confidence Interval, Analysis type: Repeated measure, All p values were calculated based on Bonferroni correction for multiple comparisons * Ancova: Adjusted for base line value KMT: Ketogenic Metabolic Therapy KD: Ketogenic Diet

Table 4) Growth factor levels in control and KMT-treated breast cancer patients

Variable	Trial arms	Baseline	Midway point	12 weeks	P value MP vs BL	P value 12 wks vs MP	P value 12 wks vs BL
Insulin (µmol/ml)	KD	9±6.6	6.1±6.7	5.7±4	0.33	1	0.03
	Control	8.2±7.3	9.1±9.6	6.9±4.5	0.81	0.22	1
Between groups	MD (95% CI)	0.82(-3.4)	-3(-8, 2.6)	-1.1(-3.1)	1	0.50	0.002*
IGF-1 (ng/ml)	KD	151±52	140±63	133±61	0.55	1	0.02
	Control	147±56	136±34	150±48	1	1	1
Between groups	MD (95% CI)	3.4(-25,33)	4.6(-29,38)	-16(-47,13)	1	1	0.77

BL: Baseline, Midway point: MP 1st follow-up or week 6 , 12 weeks or last follow-up, MD: Mean Difference, CI: Confidence Interval.

Analysis type: Repeated measure,

All p values were calculated based on Bonferroni correction for multiple comparisons

* Ancova adjusted for baseline value, weight loss and difference in calorie

KMT: Ketogenic Metabolic Therapy

KD: Ketogenic Diet IGF-1: insulin-like growth factor-1

Table 5: Influence of KMT on tumor size in locally advanced breast cancer patients after 12-week

Variable	KD	Control	p-value
Tumor size (mm) baseline[b]	54±27.6	40±27.8	**0.10[a]**
Tumor size (mm) 12-weeks[b]	27±25	34±26	**0.50***
Δ (Changes from base line)[c]	-27 (-34,-10)	-6 (-14,17)	**0.01[a]**
p-value**	0.001	0.12	

a: Calculated by independent t test

b: Data shown as mean and SD

c: mean (95% confidence interval)

* Ancova, adjusted for baseline value

**Paired T test

KMT: Ketogenic Metabolic Therapy

KD: Ketogenic diet

Table 6: Percent and frequency of TNM, LVI and PNI at 12 weeks in breast cancer patients by two trial arms

Groups		Stage					LVI		PNI	
		0	1	2	3	4	positive	negative	positive	negative
KD	No	6	3	10	3	7	5	14	3	14
	Percent	20.7	10.3	34.5	10.3	24.1	26.3	73.7	17.6	82.4
control	No	0	3	5	5	17	8	12	6	11
	Percent	0	10	16.7	16.7	56.7	40	60	35.3	64.7
P-value		0.01*					0.35**		0.26*	

* calculated by Exact Fisher

** calculated by chi square test

LVI: Lymphovascular Invasion

PNI: Perineural Invasion

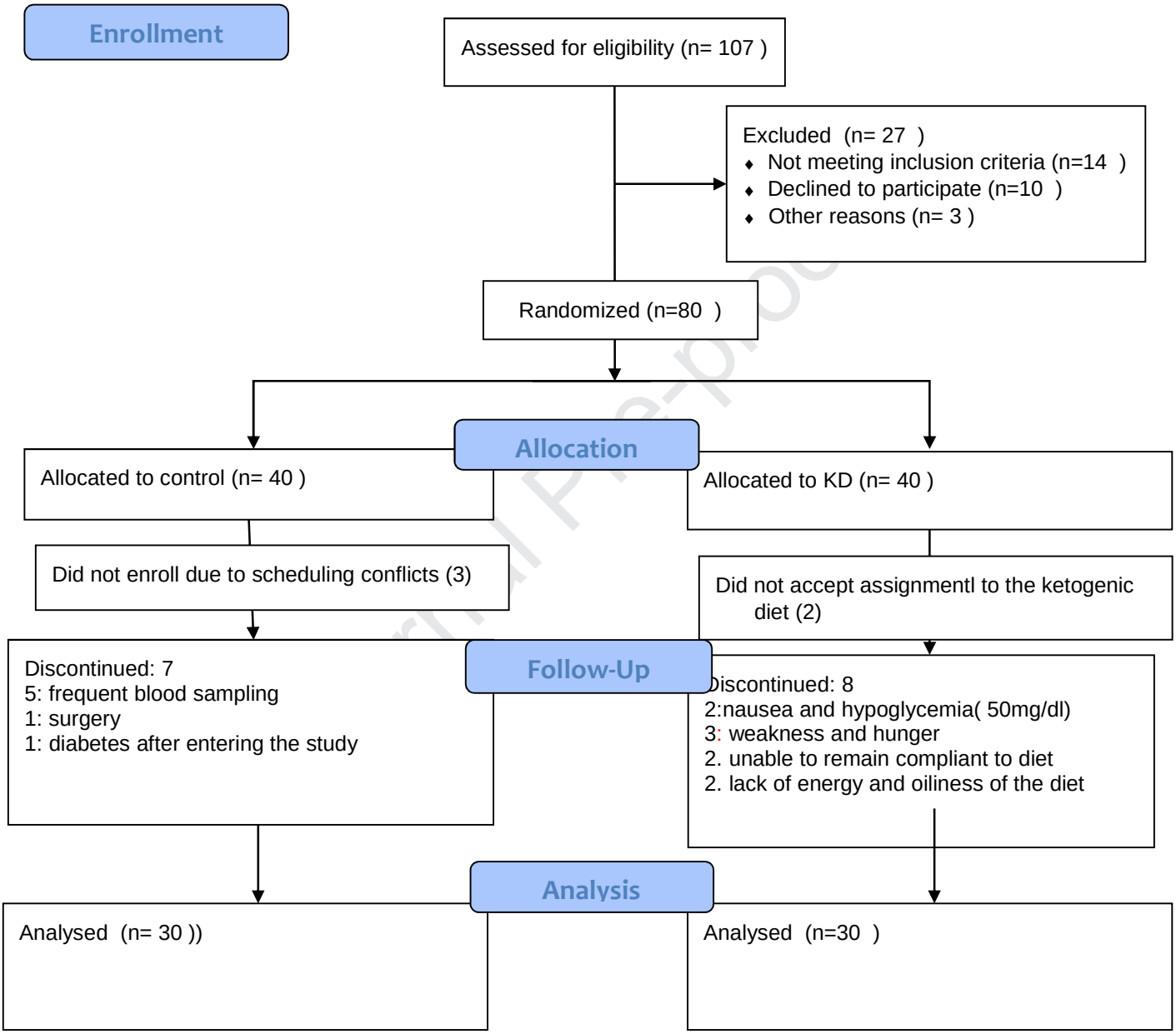

Figure 1. Flow diagram of the patient treatment process

KD: Ketogenic diet

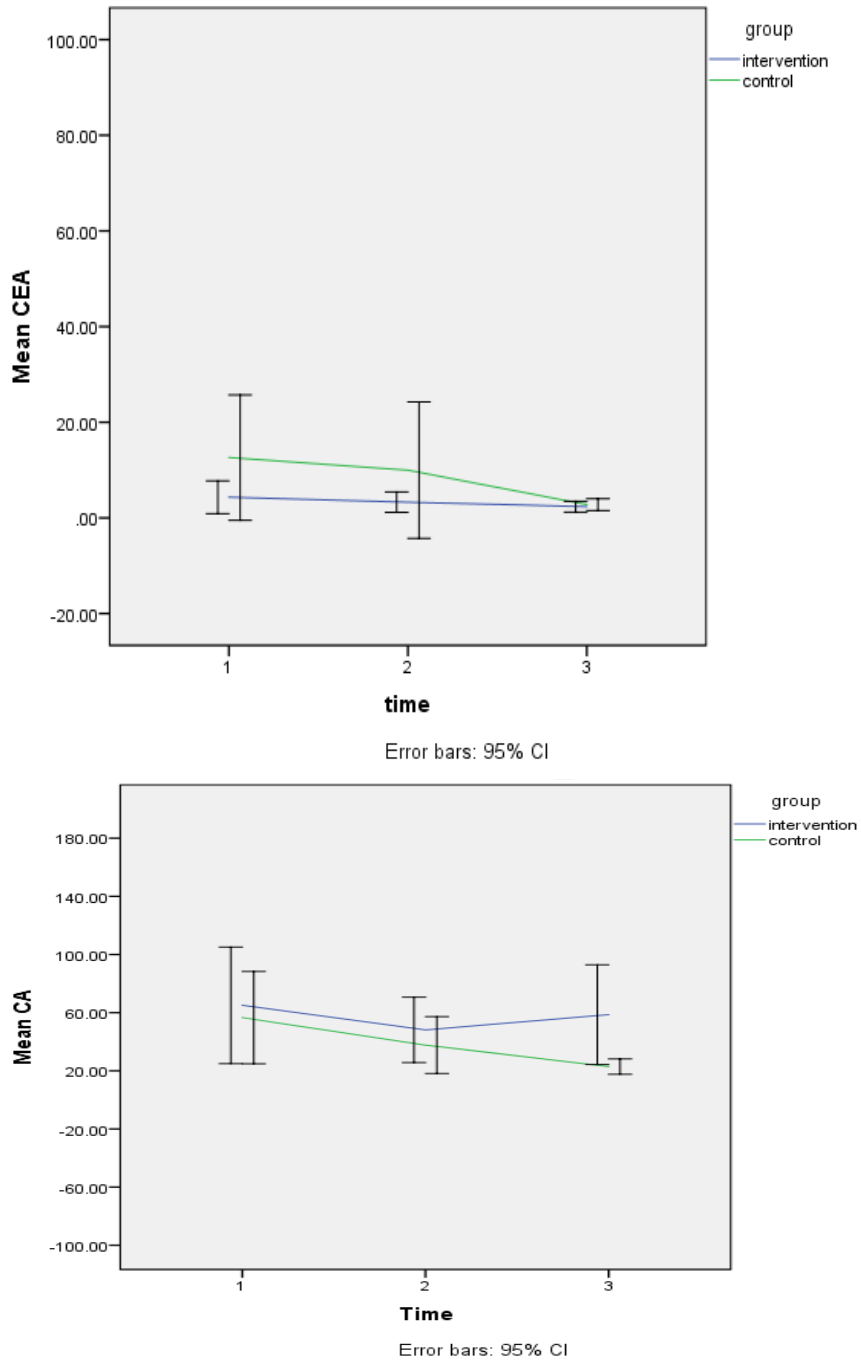

Figure 2: Comparison of trend changes in tumor marker (mean and 95%confidence interval) in breast cancer patients in two groups

Time 1: Baseline time 2: mid-point or 6-week time 3: 12-weeks

intervention: Ketogenic diet