

Influence of pre-operative oral carbohydrate loading vs. standard fasting procedure on tumor proliferation and clinical outcome in breast cancer patients – a randomized trial

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Abstract

Background The influence of carbohydrates in breast cancer is conflicting. **Objective** To determine whether preoperative per-oral carbohydrate load influences proliferation in breast tumors. **Design** Randomized controlled trial. **Setting** University hospital with primary and secondary care functions in South-West Norway. **Patients** A population-based cohort of 61 patients with operable breast cancer. **Intervention** Per-oral carbohydrate load (preOp™) 18 and 2-4 hours before surgery (n=26) or standard pre-operative fasting procedure with free consume of tap water (n=35). **Measurements** Primary outcome was post-operative tumor proliferation measured as mitotic activity index (MAI). Secondary outcomes were changes in serum insulin, insulin-c-peptide, glucose, IGF-1 and IGFBP3. Other secondary outcomes were patients' well-being and clinical outcome (median follow-up 88, range 33-97 months). **Results** In the estrogen receptor (ER) positive subgroup (n=50), high proliferation (MAI \geq 10) occurred more often in the carbohydrate group (CH) than in the fasting group (p=0.038). Progesterone receptor (PR) was more frequently negative in the CH-group (p=0.014). CH-patients had a significant between group rise in insulin (+ 24.31 mIE/L, 95% CI, 15.34 mIE/L to 33.27 mIE/L), insulin c-peptide (+ 1.39 nM, 95% CI, 1.03 nM to 1.77 nM), but reduced IGFBP3 levels (– 0.26 nM; 95% CI, – 0.46 nM to – 0.051 nM). CH-Intervention ER-positive patients had poorer relapse free survival (73%) than the fasting group (100%) (p=0.012; HR= 9.3 (95%CI, 1.1 to 77.7)). In the ER-positive patients, only tumor size (p=0.021; HR=6.07, 95%CI=1.31 to 28.03) and CH-or-fasting grouping (p=0.040; HR=9.30, 95% CI=1.11 to 77.82) had independent prognostic value. The adverse clinical outcome of carbohydrate loading occurred only in T2 patients with Relapse Free Survival of 100% in the fasting group vs. 33% in the CH-group (p=0.015; HR= inf). The CH-group reported less pain on day 5 and 6 compared to the control group (p<0.001) but showed otherwise no factors related to well-being. **Limitation** Only applicable to ER-positive breast cancer patients with T2-tumors. **Conclusions** Preoperative carbohydrate load increases proliferation and PR-negativity in ER-positive patients and worsens clinical outcome in ER-positive T2-patients.

Background

Breast cancer is the most frequent malignancy among women worldwide [1] representing 12% of all new cancer cases, and 25 % of all cancers in women [2, 3]. In Norway, its incidence has doubled during the last 50 years. Lifetime risk for a Norwegian woman of getting the disease is 10-12 % [4]. Worldwide, 570,000 women died of breast cancer in 2015, which is 15% of all cancer deaths among women [3]. Approximately 75% of all new breast cancers comprise the luminal breast cancer subtypes which express estrogen receptor (ER) and/or progesterone receptor (PR) [5]. The etiological factors of breast cancer comprise genetic, hormonal, environmental and lifestyle related elements [6]. Western lifestyle aspects risk factors including lack of physical exercise, overweight, certain hormonal and dietary factors, and diabetes mellitus type 2 have recently gained increased attention [2].

The effect of carbohydrates consumption on breast cancer incidence and outcome is probably mediated through three parallel routes. Firstly, through stimulation of the Insulin /IGF-1 axis in the epithelial breast cells, which comprises the insulin receptor (IR) [7] and the insulin like growth factor-1 (IGF1) signaling pathways [8]. This results in crosstalk of cellular signal systems and endocrine resistance in luminal breast cancers (i.e. ER-positive tumors) [9, 10]. Secondly, a substantial part of the insulin effect is mediated via tumor micro-

environmental paracrine signaling between adjacent adipocytes, fibroblasts and the epithelial cancer cell. Signaling factors like ER, IR, IGF1-R, adiponectin and leptin are involved [11]. Thirdly, alimentary glucose may also affect the cancer cells directly through the Warburg effect, which is an expedient switch that changes cellular energy metabolism from oxidative mitochondrial ATP-production to cytoplasmic aerobic glycolysis [12]. This transition enables the proliferative cancer cells to produce both ATP for energy and ribose for DNA synthesis [13].

In human breast cancer patients, there is a lack of studies on the relationship between carbohydrate/glucose content in food and quantitative insulin characteristics. Insulin is a growth factor which increases proliferation and decreases apoptosis, and elevated levels of insulin are associated with different cancers, including breast cancer [14]. Also, in breast cancer patients without diabetes, high insulin levels are associated with a poor prognosis [15]. Insulin receptors have been detected on breast cancer cells [16] although there is conflicting evidence on whether insulin directly regulates cancer proliferation, and how fast such an effect will occur. Also, there is a research deficit on the influence of carbohydrates on clinical outcome or prognostic endpoint biomarkers such as proliferation. Proliferation is often measured by the Mitotic Activity Index (MAI), Phosphohistone -H3 (PPH3) and Ki-67.[17, 18] MAI and PPH3 estimate the number of cells in the mitosis phase (M-phase) and G₂M-phase respectively, while Ki-67 detects all cells outside the G₀-phase. Notably, insulin influences the cell cycle kinetics by more rapid transit through the G₁-phase in ER-positive cells [7].

A meta-analysis has shown that in patients with abdominal surgery, administration of two per-oral carbohydrates loads administered 12 – 18 hours and 2-4 hours before elective surgery reduces postoperative insulin resistance and leads to *enhanced recovery after surgery* (ERAS) [19]. During surgery, however, breast cancer cells are known to be massively pushed into the circulation [20]. Moreover, due to the pre-operative oral carbohydrate load used in ERAS-protocols, these cells may have a much better chance of survival and of forming viable metastatic foci [21, 22]. Pre-operative oral hyperglycemic loading might bring breast cancer cells into a favorable state to escape, divide, thrive and survive during surgery, which may in turn lead to an inferior long-term prognosis for breast cancer patients [23]. It is therefore of great importance to gain more insight into the effects of administration in breast cancer regarding insulin-related characteristics, proliferation and clinical outcome.

The cell cycle in breast cancer is regarded as fast enough to be influenced by the two abovementioned ERAS-protocolled pre-operative oral carbohydrate loads [24, 25]. We chose to use MAI as our primary endpoint for proliferation. Our hypotheses were: 1. An ERAS-protocol comprising two oral carbohydrate loads will improve the post-surgical recovery of breast cancer patients; 2. The oral carbohydrate load will stimulate cellular signal systems and increase proliferation as measured by MAI; 3. A Pre-operative carbohydrate load will lead to an adverse prognosis in breast cancer patients. A subgroup analysis of ER-positive patients was planned before the study was started.

Thus, the aim of this study was to investigate whether a pre-operative carbohydrate load according to a standard ERAS-protocol influences tumor proliferation, postsurgical recovery and/or clinical outcome.

Methods

This population-based cohort of operable breast cancer patients was randomized into an intervention group receiving preoperative per-oral carbohydrate loading or to a control group comprising the standard fasting preoperative protocol with unlimited drink of water. The investigation was an open labelled study for the patient and the breast surgeon. However, all researchers at the department of pathology and the hormone laboratory were blinded for the intervention.

Patients

A total of 253 patients were assessed for eligibility between 12.05.2009 and 23.06.2010, in the catchment area of the Stavanger University Hospital in South-West Norway. The exclusion criteria were clinical or radiological T3-4 tumors at clinical examination, overt systemic metastases, ductal carcinoma in situ (DCIS), micro-invasive cancer < 2mm, or comorbidities including diabetes mellitus type I and II, Cushing syndrome, previously diagnosed cancer or being unable to co-operate in the study e.g. dementia, other serious psychiatric illnesses, language barriers or those patients not willing to sign the informed consent papers. A total of 80 patients with unequivocal operable breast cancers (Stage I and II), diagnosed by fine needle aspiration cytology (FNAC), agreed to participate in the study and were randomized (Fig.1). The last follow up date was 28.06.2017. A larger proportion of drop outs in the intervention group for various random reasons created an imbalance in numbers of patients between the allocation groups (Fig. 1).

Randomization and intervention

The randomization took place after the patients had signed the written consent to participate in the study. The randomization procedure was organized as an in-house procedure with concealed envelopes generated and distributed in two boxes by the study nurse. The allocation sequence was performed by the trial administration committee. The sequence was balanced according to age, which was performed by choosing between two boxes; one for age <55 (i.e. possible and certain premenopausal) and one for age \geq 55 (i.e. most probably postmenopausal), each with a 1:1 block randomization regarding the carbohydrate (intervention) and fasting (control) groups in each box. The surgeon in the out-patient clinic enrolled consecutively operable breast cancer patients, who agreed to participate in the trial.

Intervention

Patients who were randomized to preoperative carbohydrates drank 200 ml pre-Op™ (Nutricia, Netherlands) containing 12 % carbohydrates, 2 % glucose, and 10 % polysaccharides the evening before (i.e. 18 hours before surgery) and in the morning on the day of operation (i.e. 2-4 hours before surgery). Each patient was asked before surgery if they had been able to finish the carbohydrate drink or if they were fasting according to the randomization. The control group received standard fasting procedure with free intake of tap water.

Blinding

The study was not blinded for the patients of good reasons as the carbohydrates and tap water were impossible to keep blinded for the participants. The information on the grouping was known only for THL, who was head of the clinical part of the trial, and this information was kept in a locked safe. Other involved in

the study had no access to this information. Thus, the investigation was blinded for the laboratory personnel performing various assessments in the trial (MAI, PPH3, Ki67, Histological grading, Insulin, C-peptide etc.).

Primary treatment

The primary surgery was performed according to the recommendations of the Norwegian Breast Cancer Group (NBCG) [4] with either breast conserving treatment (BCT) or mastectomy, and sentinel node (SN) diagnostic or axillary lymph node clearance of level I and II. Adjuvant chemotherapy given was also based on the national NBCG guidelines. [4] Notably, there were no differences between the two allocation groups regarding the type of primary treatment received (Table 1).

Safety issues

The patients were hospitalized for 1-2 days after surgery. Any complications, such as hemorrhage, infection and others were recorded in the Case Report Forms. No patients died or experienced any serious complications from the received pre-operative treatment.

Blood sampling for serum analyses

Five blood samples were obtained from the participants; 1. At the time of diagnosis, 2. On admission (the day before surgery), 3. Pre-operatively before surgery, after the second pre-Op™ carbohydrate dose, 4. The day after surgery and 5. Four weeks post-surgery. Immediately after being drawn, the blood samples were put in ice water for transport to the in-house medical laboratory. The samples were spun, and the serum frozen for transport to the Hormone Laboratory, Haukeland University Hospital, Bergen, Norway. Here, insulin, insulin c-peptide, IGF-1 and IGFBP-3 were measured by the IMMULITE 2000 two-site chemiluminescent immunometric assay, Siemens Medical Solutions Diagnostics.

Histology

The tumor size was macroscopically measured in the fresh specimens following excision and cut in slices of 0.5 cm. The axillary lymph nodes from sentinel node biopsy or axillary fat from axillary dissection were first examined macroscopically by a pathologist. Then, all detectable lymph nodes were prepared for histological examination. The median number of identified lymph nodes was 3 (range 1–21, no lymph nodes detected in 2 patients). All tissues were fixed in buffered 4 % formaldehyde and embedded in paraffin. Histological sections (4 µm) were made and stained with hematoxylin–eosin–safran (HES). Histological type and grade were assessed by two pathologists (EG and JPAB) according to the World Health Organization criteria [26].

Immunohistochemistry

ER and progesterone receptor (PR), PPH3, Ki-67, and human epidermal growth factor receptor 2 (HER2), were determined by immunohistochemistry (IHC) in whole sections. Antigen retrieval and IHC techniques were based on DAKO technology as described previously [27]. Formalin fixed paraffin-embedded (FFPE) sections, 4 µm thick, serially sectioned following HES sections, were mounted onto siliconized slides (#S3002, DAKO, Glostrup, Denmark). Antigen retrieval was performed with a highly stabilized retrieval system (ImmunoPrep; Instrumec, Oslo, Norway) using 10 mM Tris/1 mM EDTA (pH 9.0) as the retrieval buffer. Sections were heated

for 3 min at 110°C followed by 10 min at 95°C then cooled to 20°C. ER (clone SP1, Neomarkers/LabVision, Fremont, CA, USA) was used at a dilution 1:400. PR (clone SP2, Neomarkers/LabVision) was used at a dilution of 1:1,000. Rabbit polyclonal anti-PPH3 (ser 10) (Upstate #06-570; Lake Placid, NY) was used at a dilution of 1:1500. Ki-67 (clone MIB-1, DAKO, Glostrup, Denmark) was used at a dilution of 1:100. All antibodies were incubated for 30 min at 22°C. The EnVision™ FLEX detection system (DAKO, K8000) was used for visualization. Sections were incubated for 5 min with peroxidase-blocking reagent (SM801), 30 min with the primary antibody, 20 min with the EnVision™ FLEX/HRP Detection Reagent (SM802), 10 min with EnVision™ FLEX DAB+ Chromogen (DM827)/EnVision™ FLEX Substrate Buffer (SM803) mix, and 5 min with EnVision™ FLEX Hematoxylin (K8008). The slides were dehydrated and mounted. All immunohistochemical stainings were performed using a Dako Autostainer Link 48 instrument and EnVision™ FLEX Wash Buffer (DM831). For HER2 assessments, DAKO HercepTest™ was used according to the procedures of the manufacturers.

Quantification of MAI, PPH3, Ki67, ER, PR, HER2, and TILs

MAI was assessed as the total number of mitotic figures counted at x400 magnification (objective 40, field diameter 450 µm at specimen level) in 10 consecutive fields of vision in the most poorly differentiated periphery of the tumor, representing a total area of 1.59 mm². Areas with necrosis or inflammation were avoided. This procedure was done as a routine diagnostic procedure, but in addition controlled by EJ as described elsewhere.[28] The PPH3 index was assessed as described elsewhere [29]. PPH3 expression was evaluated using the fully automated VIS analysis system (Visiopharm, Hørsholm, Denmark), using the same image processing principles described previously [27]. For measuring percentage of Ki-67 positive cells, the semi-automatic interactive computerized QPRODIT system (Leica, Cambridge) was used as described before [30]. For each measurement 250-350 fields of vision were randomly systematically selected, and the Ki-67 percentage was defined as [(Ki-67 positive)/ (Ki-67 positive + Ki-67 negative)] x 100. ER was scored as positive when nuclear staining was present in >1 % of the cancer cells and scored negative when <1 % of the cells were stained. PR was scored as positive when nuclear staining was present in >10 %, borderline between 1-10 % and negative when <1 % of the epithelial breast cancer cells showed nuclear staining. HER2 was scored according to the DAKO Hercep-Test scoring protocol. All 2+ and 3+ cases were regarded as positive. All sections were independently scored by two of the authors (BH and EJ). Tumor infiltrating lymphocytes (TILs) were scored semi-quantitatively in HE-stained tissue sections according to the presence or absence of stromal TILs. The relative number of TILs in the tumor stroma area was then assessed according to the method described by Salgado et al [31]. The degree of infiltration was scored in the range of 0-100 %. Positive TILs were defined as ≥10 %. Also, the tumors were classified into Luminal A (ER+/HER2-/Ki67<15%) and Luminal B (ER+/HER2-/Ki67≥15% or ER+/HER2+ regardless of Ki67) cancers according to the St. Gallen 2013 recommendations [32].

Main outcome measures

The main primary outcome measure was the difference in proliferation (measured by MAI) in the primary tumor between the study groups. The secondary outcome measures were differences in insulin related characteristics i.e. Insulin/c-peptide, IGF1 and IGFBP3 between the intervention group and control group.

Moreover, Patient Reported Outcome Measures (PROM) on the following complaints and symptoms: nausea, pain, mobilization, dizziness, insecurity and bleeding were also regarded as secondary outcomes. We applied an 'in-house' questionnaire where the patients were asked to score the six variables above on a 4-step Likert scale where 1= 'no', 2='little', 3= 'moderate' and 4 ='very much' on the 1st, 2nd, 3rd, 4th, 5th, 6th and the 7th day after the operation.

For long term outcome measures we looked at relapse free survival (RFS) defined as the time from surgery until the time the patient was diagnosed with a relapse in any location i.e. locoregional, systemic and contralateral. Breast cancer specific survival (BCSS) was defined as the time from surgery until death due to breast cancer, while overall survival (OS) was defined as the time from surgery until death of any cause. For both the primary and secondary outcomes a subgroup analysis in the ER-positive (luminal) breast cancer subtype was planned.

Statistical Analysis

Power calculation was performed on the basis of the primary endpoint. We anticipated a 20% increment in MAI in the intervention group compared to the control group. Based on the mean value of MAI in patients belonging to the catchment area of Stavanger University Hospital, [33, 34] and the reproducibility of the method to assess MAI, a total of 30 patients in each study group (i.e. 60 patients) was necessary to achieve 80% power. We decided to randomize 80 patients to allow for a 10-15 % drop-out rate.

As ER- positive breast cancer patients comprise approximately 75% of all breast cancers, there should be a reasonable number of patients to perform a subgroup analysis of the luminal breast cancers. Statistical analysis was performed with SPSS statistical software v.22 (SPSS, inc., Chicago, IL, USA). T-tests or Fishers exact test or chi square tests, as appropriate, were used to test for differences in the clinical variables between the intervention groups. Kaplan-Meier survival curves were constructed and survival differences between groups were tested by the log-rank test. The relative importance of potential prognostic variables was tested using Cox-proportional hazard analysis. In multivariable Cox regression a backward stepwise model selection procedure was used, where all covariates deemed clinically relevant were included in the initial model.

The proportion of patients reporting at least mild problems on each of the items on the PROM questionnaire (pain, nausea, mobilization, dizziness, insecurity and bleeding) on each day during the first seven postoperative days were analyzed using a mixed effects logistic regression model. Using this model, we tested for differences between the intervention and control groups. If a significant difference was found, a post hoc analysis was done by chi square tests for each of the days. We did not apply any correction for multiple testing due to the pilot and exploratory nature of the study. A two-tailed P value of 0.05 was considered as cut-off value to define the statistical significance.

Manuscript reporting

We ensure that the manuscript reporting adheres to CONSORT guidelines for reporting clinical trials, including sticking to the CONSORT check list.

Results

The various characteristics of the two allocation groups are shown in Table 1. There were 50 patients with ER-positive tumors and 11 patients with ER-negative tumors. Of the latter, 8 were HER2 negative (ER-, HER2-) and 4 were triple negative (ER-, PR-, HER2-) based on IHC-profiling. Notably, there were no differences in the distribution of the basic covariates between the carbohydrate-intervention group and the fasting group (Table 1).

Proliferation markers

In the total study cohort, none of the continuous variables MAI, Ki67 and PPH3 were different between the carbohydrate and fasting groups. However, when applying the robust and well-established prognostic threshold for MAI ($<10/\geq 10$), among the ER-positive patients ($n=50$) there were significantly more patients with high proliferation ($MAI \geq 10$) in the carbohydrate intervention (70%) than in the fasting group (30%) ($p=0.038$). (Table 2A) The same trend (58% in carbohydrate intervention) compared to the control group (42%, fasting group) was found ($p=0.083$) when all tumors were considered. Moreover, in lymph node negative luminal patients the same correlation was stronger with a Kendall's tau-b $r = 0.488$ ($p=0.017$) and Gamma $r = 1.000$ ($p=0.017$). Pearson Chi square = 7.62 ($p=0.006$; Fischer exact = 0.014 (two sided)) (Table 2B).

Progesterone Receptor

In the carbohydrate group, there were significantly more patients with PR-negative tumors (50%) than PR-positive tumors (20%) compared to the fasting group. ($p=0.014$), independent of Luminal A/B status.

Serum glucose and insulin responses.

Response to the preoperative carbohydrate loading was assessed by the difference between the pre-operative serum values and the values taken at admission (i.e. serum levels after carbohydrate loading minus fasting baseline values in both groups) (Table 5). As expected, the intervention group had a significant increment in both S-Insulin ($+24.31$ mIE/L, $p < 0.0001$, 95%CI, 15.34 mIE/L to 33.27 mIE/L) and S-insulin c-peptide ($+ 1.39$ nM, $p < 0.0001$; 95%CI, 0.21nM to 0.97nM). The upper quartile (Q_4) border value of 2.40 nM was equal to the upper value of the normal range of insulin c-peptide (Table 5), indicating that 25% of the patients had c-peptide values compatible with insulin resistance. Regarding IGFBP3, there was a significant reduction after carbohydrate loading within the intervention group of $- 0.43$ nM ($p<0.0001$, 95%CI, $- 0.56$ nM to $- 0.27$ nM) and also compared to the control group ($- 0.26$, $p=0.015$, 95%CI, $- 0.46$ nM to $- 0.051$ nM) (Table 5). There were no changes in S-glucose and S-IGF-1 values within or between the two study groups (Table 5).

The changes in the various variables from Table 5 are depicted in Figure 5 A-F.

Quality of Life data

In the carbohydrate intervention group, fewer patients reported mild and moderate pain during the first seven postoperative days than in the fasting group ($p<0.001$) which in post hoc analysis was significant on postoperative day 5 (28% vs 47%; $p=0.038$) and day 6 (28% vs 50%; $p < 0.001$). Otherwise, there were no

significant differences between the two groups regarding the other items from the PROM-questionnaire (nausea, mobilization, dizziness, insecurity and bleeding) (data not shown).

Long term clinical outcome

Median follow-up time for RFS was 88 months (range 33 to 97) and for BCSS the median was 88 months (range 45 to 97). There were 8 patients who experienced a relapse; loco-regional (n=1), systemic (n=6) and contralateral (n=1) and 5 patients died of breast cancer.

Relapse free survival

Randomization to intervention with preoperative carbohydrates was found to have a weak and borderline influence on RFS when analyzed in the whole study cohort (Table 3). However, in the ER positive patients who received carbohydrates preoperatively a reduced RFS of 71% compared to 97% in the control group ($p=0.012$, $HR=9.3$, $CI=1.1$ to 77.7 ; Table 3 and Fig.2A) was observed. The covariates tumor diameter between 2 and 5 cm (T2), and the proliferation marker Ki67 (both $\geq 15\%$ and $\geq 30\%$) had a significant negative influence on RFS in both the whole group and in the ER-positive cohort (Table 3). In the ER-negative subgroup there was no influence of the carbohydrate /fasting grouping on RFS (Fig. 2B). The following co-variables were deemed clinically relevant to adjust for: tumor size (T), nodal status (N), Histological grade, PR-status, HER2-status, Ki67-15%, Ki67-30%, PPH3-13, MAI-10, TILs, Luminal A/B status, Carbohydrate /Fasting grouping, Chemotherapy, Radiotherapy and Endocrine therapy, BMI-75p, BMI-25 and smoking status. In the multivariable analysis, tumor size (T1/T2) ($p=0.021$; $HR=6.07$, $95\%CI=1.31$ to 28.03) and Carbohydrate/Fasting grouping ($p=0.040$; $HR=9.30$, $95\%CI=1.11$ to 77.82) were the only two variables left in the final Cox model. As T2 tumors were more frequent in the intervention group we performed a Kaplan Meier analysis of the influence of the carbohydrate intervention on RFS stratified on T1 vs T2. This analysis showed that the unfavorable prognostic effect of the carbohydrate loading was not present in the T1 (≤ 2 cm) patients but was strongly prognostic in the T2 patients (Fig. 2C and 2D). In the T2 group, the carbohydrate loaded, and fasting patients had a RFS of 33 % and 100% respectively ($p=0.031$; $HR=inf$). In the T2 subgroup there was a significantly higher mean serum level of preoperative insulin c-peptide among the patients who experienced a relapse versus those who were relapse free (2.02 nM vs. 0.838 nM respectively, $p=0.025$). Notably, there was an even distribution of Luminal A and Luminal B tumors among the patients comprising T2 tumors who experienced a relapse versus those who did not ($p=0.47$).

Breast Cancer Specific Survival

In the unadjusted analysis of BCSS, intervention with carbohydrates showed a significantly inferior BCSS in ER-positive patients compared to the control group (Table 4; Fig. 3A). In ER-positive T2 tumors the carbohydrate intervention group had the worst BCSS of 30 % compared to 100% in the control fasting group ($p=0.031$, $HR=infinite$, due to zero relapses in one of the two groups) (Fig. 3B). In addition, tumor size, nodal status and Ki67-30% provided significant prognostic information in the unadjusted analysis (Table 4.) In the multivariable analysis, only Ki67-30 remained in the final model. In general, the small number of patients and endpoints hampered a robust multivariable analysis.

Overall Survival

The univariate analysis of overall survival (OS) of ER+ patients showed only a borderline significance of OS for the Carbohydrate group (81%) compared to the fasting group (99%) ($p=0.068$; HR=6.02; 95%CI=0.672 – 53.8) (Fig.4A). Only tumor size remained as explanatory factor in the final Cox model (HR=17.1; 95% CI =17.1 – 153). In the ER+/T2 patients the corresponding OS was 33% vs 100% respectively ($p=0.031$; HR=inf) (Fig. 4B). In the Cox model, Carbohydrate/Fasting status entered into the last step, but the model was considered too unstable for a reliable report.

Adverse events

No adverse events were seen in neither of the two study arms. Especially, no signs of pathologic elevated fasting blood sugar levels (i.e. > 6 mmol/L) was seen in the two groups. Furthermore, in the carbohydrate arm no signs of occult diabetes mellitus was seen, i.e. blood sugar levels > 10 mmol/L after carbohydrate loading.

Discussion

Glucose has been correlated with cancer for nearly one century. Warburg (1925) was the first to describe the phenomenon, that cancer cells have a much stronger tendency to take up glucose [35], for which he received the Nobel prize in 1932 (amongst other findings) [36]. However, to our knowledge the current study is the first prospective randomized trial to evaluate the effects of preoperative carbohydrate loading on tumor proliferation and (short term vs. long term) outcome in operable breast cancer patients. In patients with ER-positive tumors (i.e. luminal tumors), significantly more patients with $MAI \geq 10$ were observed in the carbohydrate intervention than in the fasting group. Luminal cancers have, on average, a lower proliferation rate than ER-negative and triple negative cancers [37]. As such, the proliferation increasing effect of carbohydrate loading in luminal cancers understandably leads to a higher percental increase of patients crossing the prognostically essential MAI-10 threshold. Most ER-/triple negative breast cancer patients already have a MAI greatly exceeding 10. Therefore, carbohydrate loading will probably not increase proliferation clinically significantly as they have an 'a priori' high risk for distant metastases [38]. In addition, the Luminal A patients exposed to excess carbohydrates may turn into Luminal B tumors and thereby statistically increase their risk for recurrences. This is in agreement with the fact that luminal breast cancers respond directly to circulating insulin increase through altered transmembrane insulin receptors (IR) [39]. Thus, in the present study, the observation of an increase in insulin /c-peptide in the intervention group could explain the increased MAI and Ki67 in the ER-positive group. Likewise, as triple negative cancers better utilize the IGFBP3-pathway in EGF1-signalling [40], our observed *reduction* in IGFBP3 after the carbohydrate loading may account for the lack of response to proliferation in the ER-negative group. This could suggest, that the differential responses to the I/IGF1 axis between luminal and triple negative cancers [41] may explain our observed differences in response to per-oral carbohydrate loading and mitotic activity between the ER-positive and ER-negative group.

The observed inferior RFS in the ER-positive T2 tumors and not in the T1 tumors in the present study suggests that larger tumor size may influence to what extent the cancer cells have activated all necessary features to promote the epithelial-mesenchymal transition (EMT) process [42], and seed out micro

metastases. These processes turn into clinically overt relapses after some years [43]. This is in line with other research, which has found a positive correlation between tumor size and relapse [44], and also between tumor size and development of endocrine resistance [45]. A crucial question is, to what extent the preoperative carbohydrate load to the patients in the present study has promoted the EMT-process in the T2-T3 tumors and thus created more micro metastases [46, 47]. Importantly, increased signaling through the Insulin/IGF axis is known to promote both the EMT-process [48] and chemotaxis [49], which increase the risk for minimal residual disease to occur. Furthermore, the preoperative carbohydrates may have been administered in a critical time window of the cancer's life cycle. The amount of liberated circulating tumor cells (CTCs) from the primary tumor is known to sharply increase during surgery [50]. Thus, the administered carbohydrates may have given such CTCs a systemic biological support from a triple survival benefit through the Warburg effect [12], the insulin /IGF-1 axis [51] and the paracrine signaling with distant located adipocytes [11]. Furthermore, increased IR/IGF signaling promotes the protein synthesis in the same way the PR-pathway does. Consequently, the upregulation of IR/IGF-signaling will suppress transcription of PR in the cell [52], which is considered to be part of the endocrine switch. Moreover, dietary carbohydrates may also down regulate the gene expression of PR through epigenetic mechanisms [53]. These mechanisms support our finding with less PR-positivity in the carbohydrate arm. All together, these components of the endocrine switch make CTCs more resilient to the adjuvant endocrine treatment that follows surgery [9, 54]. The present study seems to support the novel principle of manipulation of the perioperative nutrient status for adjuvant treatment purposes. Recently, the complete opposite situation with a postoperative low carbohydrate / ketogenic diet has been advocated in pancreatobiliary cancer surgery as an adjuvant anti-cancer therapy option [55].

The fact that the distribution of larger tumor size was skewed to the 'Carbohydrate group' (even though not significantly), there might be another explanation of this observation than statistical chance. As the carbohydrates affected proliferation, they may also have affected the growth of the tumor cells in the periphery of tumor (were the MAI in fact is measured). This may have resulted in more blurry demarcations of the tumor, which interferes with the accuracy of tumor size measurement. Thus, the increased tumor size in the carbohydrate group may have tumor-biological reasons.

The observed inferior prognosis of patients who received carbohydrate load and comprise T2 tumors calls for some reflection. Patients with higher levels of insulin c-peptide may be more responsive not only to the carbohydrate loading they received in the present study, but also to carbohydrates in every meal they consume during the period of adjuvant therapies, and hereafter. These patients may comprise a subclinical insulin resistant state, which is known to be a risk factor for relapsing from breast cancer in non-diabetic women [56]. Thus, it may be that tumor size combined with insulin c-peptide status may predict an increased effect of adjuvant metformin or other insulin lowering drugs in the adjuvant treatment of breast cancer patients. Metformin attenuates the systemic biological effect of IR /IGF on tumor promoting signaling by improving insulin sensitivity and suppressing liver glucose output, which leads to reduced levels of systemic circulating insulin [14]. This will further mitigate paracrine signaling, overcome endocrine resistance [51, 57] and improve prognosis in breast cancer [58-61]. The present study supports the hypothesis that adjuvant metformin or other insulin-lowering therapeutic interactions may have their largest effect in breast cancer patients with ER-positive T2 tumors. In addition, the greatly increased glucose consumption of cancer cells measured by positron emission tomography (PET) with ¹⁸F-deoxy-glucose (FDG) as tracer [62] identifies

patients with an inferior clinical outcome [63]. This may also serve as a promising proxy for becoming an insulin / metformin responder.

The effect of the carbohydrate loading on well-being in the present study had a very limited clinical subjective effect (i.e., only reduced pain on the 5th and 6th day after surgery). Of note, no difference in mobilization and hospitalization was found. This is most probably due to the short duration of the operation and the extraperitoneal nature of the surgical procedure in breast cancer patients. Recently, the health authorities in Norway have introduced new national guidelines for a more standardized trajectory in breast cancer [64]. Also, day-care surgery comprising anesthesiologic medication with a short half-life leading to less side effects for the patients and optimization of pain relief regimen has been introduced since this trial was performed. Thus, the present study does not support introducing carbohydrate loading in this patient group, especially due to the worrying inferior relapse free survival observed in the carbohydrate intervention group.

The strengths of the above described biological model are, that it allows for assessing changes in the breast tumor after manipulating the metabolic environment preoperatively, and thus combines assessment of primary tumor characteristics in concert with systemic metabolic changes. Also, the stable nature of insulin c-peptide has compensated for the more short-lived insulin and IGF. This may explain the more robust nature of insulin c-peptide in the various analyses.

There are several weak points in the present study. The number of patients in the intervention arm turned out to be lower than calculated in the power analysis. This may have introduced a type II error in the various statistical analyses. Furthermore, the low number of events and patients at risk in the various survival analyses must call for caution in the interpretation of the results. Moreover, the unbalanced number of participants in the carbohydrate group and the fasting group may have introduced confounders. However, as all basic characteristics were evenly distributed between the two study arms, the risk for such confounders is probably quite low. Also, the proportion of missing data was very low, which contribute to strengthen the study. Regarding tumor markers, a preoperative biopsy of the tumor would have turned the patients into their own controls. Thus, we could have addressed several questions raised in the discussion, e.g. the increased PR-negativity in the carbohydrate group. In future studies, preoperative biopsy must be included to improve the internal validity of the trial.

Finally, the external validity of the present study will be limited to luminal breast cancers with T2 tumors. Thus, the present study should be expanded in a multicenter manner, but only in luminal type breast cancers without the PROM QoL-questionnaire. Moreover, a high insulin c-peptide response to a carbohydrate load may predict being at high risk for relapse. Future research should pursue this clue by adding metabolomic studies in the further search for predictive circulating predictive/prognostic biomarkers for systemic relapse in the minutest state possible [65].

Conclusions

The goal of this study was to investigate the influence of carbohydrates on the biological characteristics of breast cancer. Our working hypothesis was that preoperative carbohydrate loading may affect proliferation as well as clinical outcome. In the carbohydrate-loading group, the levels of insulin and insulin-c-peptide were

increased while those of IGFB3 were decreased. We found that there were more ER+ patients with a MAI ≥ 10 among patients who received preoperative carbohydrate loading compared to those who fasted. In addition, the proportion of PR- patients was higher in the carbohydrate group. In ER+ patients with tumors larger than 2 cm (T2), carbohydrate loading seemed to affect clinical outcome with significantly decreased relapse-free survival (RFS), breast cancer-specific survival, and overall survival. Only RFS had enough events to enter into a Cox regression model of which carbohydrate/fasting status and tumor size were the only independent explanatory factors. However, because this study was not powered for survival outcomes, these analyses must be regarded as suggestive. In addition, caution is needed when interpreting the results due to the small sample size and relatively short follow-up. Intriguingly, the decreased expression of PR in the carbohydrate-loaded group suggests the development of endocrine resistance through signaling via membrane-bound receptors, opening up another possibility for the reduced clinical outcome than increased proliferation. In conclusion, the results of this study indicate that peroral carbohydrates given preoperatively may influence both systemic and tumor biology to the benefit of breast cancer cells. Thus, explorative metabolic investigations are warranted that focus on identifying novel biomarkers associated with the observed impaired clinical outcome.

Abbreviations

ATP: adenosine triphosphate

BCSS: breast cancer specific survival

BCT: breast conservative therapy

CI: confidence interval

CTC: circulating tumor cell

DCIS: ductal carcinoma in situ

EGFR: epidermal growth factor receptor

EMT: epithelial mesenchymal transition

ER: estrogen receptor

ERAS: enhanced recovery after surgery

FNAC: fine needle aspiration cytology

HER2: human epithelial growth factor receptor 2

HES: haematoxyllin-eosine saphron staining

HR: hazard ratio

IGF-R: insulin like growth factor receptor (gene)

IGF-R: insulin like growth factor receptor (protein)

IGF1: insulin like growth factor 1

IGF1R: insulin like growth factor 1 receptor

IHC: Immunohistochemistry

IR: insulin receptor

MAI: mitotic activity index

MRI: magnet resonance imaging

NBCG: Norwegian breast cancer group

NSD: Norwegian center for research data

PPH3: phosphorylated phosphohistone 3

PR: progesterone receptor

PROM: patient reported outcome measure

RFS: relapse free survival

SN: sentinel node

TIL: tumor infiltrating lymphocytes

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

The randomized trial was approved by the Regional Ethics Committee (Accession number 2015/1445), NSD (Norwegian Centre for Research data) (# 20984) and "The Norwegian Biobank registry (# 2239). An informed consent form was signed by each patient. The trial was retrospectively registered in Clinicaltrials.gov (NCT03886389). The reason for delayed registration was that we were not aware of the obligation to register prospectively at the time.

Consent for publication

Not applicable

Availability of data and material

The data that support the findings of this study are available from Stavanger Breast Cancer Research Group, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission from Stavanger Breast Cancer Research Group.

Manuscript reporting

We ensure that the manuscript reporting adheres to CONSORT guidelines for reporting clinical trials, including filling out the CONSORT check list.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

THL included and operated all of the patients, building the database, contributed to statistical analyses, interpretation of data. MA contributed to quality assurance of the database. AV performed the drawing of all the blood samples and laboratory analyses. IS contributed to the laboratory analyses. EG performed surgical pathological analysis with histological grading and morphological analysis of the tumor. JTK gave expert advice on the statistical analyses. LAA contributed with scientific support and advice. HS contributed to the concept of the study, statistical analyses, interpretation of data, and funding of the study. EAM contributed to the concept of the study, performed the assessment of the pathological parameters and scorings and interpretation of data. JPAB contributed to the concept of the study, the analysis and interpretation of the data.

All co-authors contributed to writing the manuscript and gave their final approval of the last version to be published.

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Authors' information (optional)

N.A.

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Tables

Table 1. Baseline characteristics of patients in the two study groups

Variable	Carbohydrate group (n=26) n (%)	Missing data (Intervention Group)	Fasting group (n=35) n (%)	Missing data (Control group)	P
Age					
<55	12 (46)	0	16 (46)	0	0.973
>55	14 (54)	0	19 (54)	0	
BMI (kg/m²)	25.0 (3.9)	4	25.1 (3.0)	3	0.868
BMI < 25*	14 (64)	4	17 (53)	3	0.443
BMI ≥ 25	8 (36)		15 (47)		
BMI < 75 percentile **	18(82)	4	23 (76)	3	0.401
BMI ≥ 75 percentile	4(18)		13(24)		
Menopausal status					
Premenopausal	4 (17)	1	7 (22)	1	0.627
Postmenopausal	20 (83)	1	25 (78)	2	
HRT - yes	8 (35)	3	10 (32)	4	0.937
HRT – no	14 (61)		19 (61)		
HRT- not relevant	1 (4)		2 (7)		
HRT use (Years)	4.7 (4.3)	16	7.9 (5.8)	25	0.176
Tumor size (mm)	19.4	0	15.0	0	0.094
Tumor category					
T1	16 (62)	0	29 (83)	0	0.061
T2	10 (38)	0	6 (17)	0	
Histological Grade #					0.157
1	4 (15)	0	7 (20)	0	0.852
2	10 (39)	0	20 (57)	0	
3	12 (46)	0	8 (23)	0	
pN negative	18 (69)	0	25 (71)	0	0.843
pN positive	8 (31)	0	10 (29)	0	
Number LNs removed	5.5	2	5.8	0	0.191
Number positive LNs	0.38	2	0.86	0	
Estrogen receptor					
Positive (≥1%)	21 (81)	0	29 (83)	0	0.834
Negative (<1%)	5 (19)	0	6 (17)	0	
Progesterone receptor					
Positive (≥ 10%)	13 (50)	0	28 (80)	0	0.014
Negative (<10%)	13 (50)	0	7 (20)	0	
HER2					
Positive	3 (12)	0	1 (3)	0	0.176
Negative	23 (88)	0	34 (97)	0	
MAI (median, IQR)	7 (2-9)	1	5 (2-9)	0	0.647
MAI < 10	14 (56)	1	27 (77)	0	0.083
MAI ≥ 10	11 (44)		8 (23)	0	
Ki67 (mean, SD)	30.4 (28.2)	0	28.0 (26.5)	1	0.747
Ki67 < 15%	9 (35)	0	17 (50)	1	
Ki67 ≥ 15%	17 (65)	0	17 (50)	0	0.233

Ki67 < 30%	14 (54)	0	24 (71)	1	0.182
Ki67 ≥ 30%	12 (46)	0	10 (29)	0	
PPH3 (mean, SD)	20.2 (24.7)	0	20.5 (26.9)	0	0.966
PPH3 < 13	14 (54)	0	21 (60)	0	0.631
PPH3 ≥ 13	12 (46)	0	14 (40)	0	
TILs (mean %, SD)	4.7 (10.7)	0	4.3 (7.3)	1	0.137
TILs					
Positive (>10%)	2 (8)	0	4 (11)	0	0.663
Negative (<10%)	24(92)	0	31(89)	0	
Luminal type ¶					
Luminal A	16 (62)	0	23 (66)	0	
Luminal B	10 (38)	0	12 (34)	0	0.737
Glucose					
Admission	5.4 (1.1)	0	5.3 (0.6)	0	0.864
Preoperative §	5.2 (1.8)	0	5.1 (0.6)	0	0.739
S-Insulin					
Admission	9.4 (8.5)	0	9.1 (6.6)	0	0.886
Preoperative §	33.7 (20.2)	0	9.1 (5.9)	0	<0.0001
S-insulin-c-peptide					
Admission	0.69 (0.32)	0	0.75 (0.32)	0	0.517
Preoperative §	2.10 (1.05)	0	0.75 (0.27)	0	< 0.0001
Surgery					
BCT	15 (58)	0	23 (66)	0	
Mastectomy	11 (42)	0	12 (34)	0	0.523
Axillary staging					
SN	21(81)	0	28 (80)	0	
ALND	5 (19)	0	7 (20)	0	0.940
Reoperation - 1					
-Breast	1 (20)	0	1(50)	0	
-Axilla	4 (80)	0	1(50)	0	0.427
Chemo therapy					
Yes	12 (46)	0	17 (47)	0	
No	14 (53)	0	18 (51)	0	0.852
Radiation therapy					
Yes	17(68)	0	26 (74)	0	
No	8 (32)	1	9 (26)	0	0.594
Endocrine therapy					
Yes	17(65)	0	22 (63)	0	
No	9 (35)	0	13 (37)	0	0.839
Smoking status		5		4	
-Never smoked	5 (24)		10 (32)		0.650
-Former smoker	9 (43)		14 (45)		
-Ongoing smoking	7 (33)		7 (23)		

* BMI-25 represents a dichotomized BMI < 25 or ≥ 25 on the BMI scale.

** BMI-75p represents a dichotomized BMI with cut off < /≥ 75 percentile i.e. </≥ 26.8 on the BMI scale.

Tumor size category analyzed as T1 vs T2.

The histological grading is performed according to the Nottingham algorithm.

¶ Luminal A= ER+/HER2-/Ki67<15% & Luminal B=ER+/HER2-/Ki67≥15%.

|| Blood samples taken at fasting state at the time patients were admitted in the hospital approx. 24-30 hours before surgery.

- Preoperative blood samples at taken 1-2 hours before the surgical procedure commenced.

BMI, body mass index; HRT, hormonal replacement therapy; pT, pathological tumor size in mm or category; pN: pathological lymph node status; LN, lymph node; HER-2, human epidermal growth factor receptor 2; MAI, mitotic activity index; TILs, tumor infiltrating leucocytes; PPH3, phosphorylated phospho-histone 3; SN, sentinel node; ALND, axillary lymph node dissection.

Table 2A.

Cross table MAI and allocation groups in ER+ patients

			Carbohydrate	Fasting	Total
	MAI<10	Count	13	26	39
		%	65.0%	89.7%	79.6%
	MAI≥10	Count	7	3	10
		%	35.0%	10.3%	21.4%
Total		Count	20	29	49
		%	100.0%	100.0%	100,0%

Pearson Chi-square: 4.430, df=1, p=0.035.

Fischer exact: 0.041 (one sided) and 0.068 (two sided).

r (gamma) = 0.647 (p=0.042).

r (Kendall's tau-b) = 0.301 (p=0.042).

Table 2B.

Cross table MAI and allocation groups in ER+ /LN negative patients

			Carbohydrate	Fasting	Total
	MAI<10	Count	8	20	28
		%	66.7%	100.0%	87.5%
	MAI≥10	Count	4	0	4
		%	33.3%	0.0%	12.5%
Total		Count	12	20	49
		%	100.0%	100.0%	100.0%

Pearson Chi-square: 7.619, df=1, p=0.006.

Fischer exact: 0.014 (one sided) and 0.014 (two sided).

r (gamma) = 1.000 (p=0.017)

r (Kendall's tau-b) = 0.488 (p=0.017)

Table 3. Univariable analysis of Relapse Free Survival

	Whole cohort (n=61)				ER positive patients (n=50)			
Characteristics	Event / at risk (% survival)	Log rank P	HR	95 % CI	Event / at risk (% survival)	Log rank P	HR	95 % CI
Preoperative randomization								
Fasting Carbohydrates	2/35 (94) 6/26 (77)	0.049	1 4.4	0.9 to 21.7	1/29 (97) 6/21 (71)	0.012	1 9.3	1.1 to 77.7
Nodal status								
N0	3/43 (93)		1		3/33 (91)		1	
N+	5/18 (13)	0.03	9.8	1.10 to 88.1	4/17 (77)	0.16	2.8	0.63 to 12.6
Tumor size								
T1	3/45 (93)		1		3/39 (92)		1	
T2	5/16 (69)	0.009	5.5	1.3 to 23.2	4/11(64)	0.008	6.0	1.3 to 27.0
Nottingham grade #		0.33				0.31		
Grade 1	0/11 (100)		1		0/11 (100)		1	
Grade 2	5/30 (83)	-	Inf.	Inf.	5/30 (83)		Inf.	Inf.
Grade 3	3/20 (85)	-	inf.	Inf.	2/9 (78)		inf.	Inf.
Estrogen receptor								
Positive (≥ 1%)	7/50 (86)		1		-		-	-
Negative (<1%)	1/11 (91)	0.67	1.6	0.2 to 12.7	-		-	-
Progesterone receptor								
Positive (≥10%)	4/41 (37)		1		3/37 (92)		1	
Negative (< 10%)	4/20 (80)	0.27	2.1	0.5 to 8.6	4/13 (69)	0.048	4.0	0.90 to 18.1
HER2								
Negative	7/57 (88)		1		6/49 (88)		1	
(0 to 1+)								
Positive	1/4 (75)	0.46	2.1	0.3 to 17.5	1/1 (0)	0.005	11.7	1.3 to 105.1
(2+ to 3+)								
MAI								
< 10	5/41 (88)		1		4/39 (90)		1	
≥ 10	3/19 (66)	0.66	1.4	0.3 to 5.8	3/10 (70)	0.09	3.4	0.8 to 15.2
MAI								
< 3	2/16 (88)		1		2/16 (88)		1	
≥ 3	6/44 (86)	0.89	1.1	0.2 to 5.5	5/33 (85)	0.80	1.2	0.2 to 6.4
PPH3								
< 13	3/35 (91)		1		3/35 (91)		1	
≥ 13	5/26 (81)	0.26	2.2	0.5 to 9.4	4/15 (73)	0.12	3.1	0.7 to 14.0
Ki67								
< 15	0/26 (100)		-	-	0/25 (100)		1	
≥ 15	8/34 (77)	0.008	-	-	7/24 (71)	0.003	*	*

Ki67								
< 30	3/38 (92)		1		3/37 (92)		1	
≥ 30	5/22 (77)	0.093	3.2	0.8 to 13.4	4/12 (67)	0.023	4.8	1.1 to 21.8
TILs								
Negative (<10%)	2/13 (85)				7/55 (87)		1	
Positive (≥10%)	6/48 (88)	0.77	1.4	0.2 to 3.9	1/6 (83)	0.75	2.2	0.24
Luminal status ¶								
Luminal A	3/39 (92)				2/28 (93)			
Luminal B	5/22 (77)	0.091	3.2	0.77 to 13.5	5/22 (77)	0.11	3.5	0.68 to 18.1
Chemotherapy								
Yes	6/29 (79)		1		5/20 (75)		1	
No	2/32 (94)	0.096	0.28	0.06 to 1.4	2/30 (93)	0.069	0.25	0.05 to 1.3
Radiotherapy								
Yes	6/43 (86)		1		5/38 (87)		1	
No	2/17 (88)	0.90	0.91	0.18 to 4.5	2/12 (83)	0.72	1.4	0.26 to 7.0
Endocrine Therapy								
Yes	7/39 (82)		1		6/36 (83)		1	
No	1/22 (96)	0.15	0.24	0.03 to 2.0	1/14 (93)	0.38	0.40	0.05 to 3.3
BMI-25 §								
< 25	3/31 (90)		1		3/26 (89)		1	
≥ 25	4/23 (83)	0.40	1.9	0.42 to 8.4	3/20 (85)	0.70	1.4	0.28 to 6.8
BMI-75p 								
< 75p	4/41 (90)		1		4/36 (89)		1	
≥ 75p	3/13 (77)	0.201	2.57	0.57 to 11.5	2/10 (80)	0.417	1.99	0.36 to 10.9
Smoking								
-Never smoked	4/15 (73)		1		3/12 (87)		1	
-Former smoker	1/23 (96)		0.22	0.025 to 2.00	1/20 (95)		0.26	0.027 to 2.5
-Ongoing smoking	1/14 (93)	0.065	0.14	0.015 to 1.22	1/12 (92)	0.15	0.17	0.017 to 1.6

*HR (95% CI) was not computed as the equation did not converge as no events occurred in one or more categories.

The histological grading is performed according to the Nottingham algorithm.

¶ Luminal A= ER+/HER2-/Ki67<15% & Luminal B=ER+/HER2-/Ki67≥15% or ER+/HER2+.

- BMI-25 represents a dichotomized BMI < 25 or ≥ 25 on the BMI scale.

|| BMI-75p represents a dichotomized BMI with cut off < /≥ 75 percentile i.e. </≥ 26.8 on the BMI scale.

BMI, body mass index; HRT, hormonal replacement therapy; T, tumor size in mm or category; N: pathological lymph node status; LN, lymph node; N0, node negative; N+, node positive (assessed by pathologists); HER-2, human epidermal growth factor receptor 2; MAI, mitotic activity index; TILs, tumor infiltrating leucocytes; PPH3, phosphorylated phospho-histone 3.

Table 4. Univariable analysis of Breast Cancer Specific Survival

	Whole study cohort (n=61)				ER-positive patients (n=50)			
Variable	Event / at risk (% survival)	Log rank P	HR*	95 % CI	Event / at risk (% survival)	Log rank P	HR*	95 % CI
Preoperative randomization								
Fasting Carbohydrates	1/35 (97) 4/26 (85)	0.086	1 4.4	0.88 to 21.7	0/29 (100) 4/21 (81)	0.015	1 *	*
Nodal status								
N0	1/43 (98)				1/33 (82)		1	
N+	4/18 (78)	0.012	4.4	1.05 to 18.5	3/17 (82)	0.080	2.80	0.63 to 12.6
Tumor size			-	-				
T1	0/45 (100)		1		0/40 (100)		1	
T2	5/16 (69)	<0.0001	5.5	1.32 to 23.1	4/10 (60)	<0.0001	*	*
Nottingham grade #		0.556				0.352		
Grade 1	0/11 (100)		1		0/11 (100)		1	
Grade 2	3/30 (90)		*	*	3/30 (90)		*	*
Grade 3	2/20 (90)		*	*	1/9 (89)		*	*
Estrogen receptor					-	-	-	-
Positive (≥ 1%)	4/50 (92)		1		-	-	-	-
Negative (<1%)	1/11 (91)	0.852	0.64	0.079 to 5.21	-	-	-	-
Progesterone receptor								
Positive (≥10%)	4/41 (90)		1		3/37 (92)		1	
Negative (<10%)	1/20 (95)	0.543	0.51	0.057 to 4.59	1/13 (92)	0.94	0.93	0.1 to 8.9
HER2								
Negative (0 to 1+)	4/57 (93)		1		3/49 (94)		1	
Positive (2+ to 3+)	1/4 (75)	0.248	3.37	0.38 to 30.2	1/1 (0)	0.001	11.7	1.31 to 105.1
MAI								
< 10	3/41 (93)		1		3/39 (92)		1	
≥ 10	2/19 (90)	0.645	1.5	0.25 to 9.1	2/10 (80)	0.23	4.1	0.6 to 29.3
MAI								
< 3	1/16 (94)		1		1/16 (88)		1	
≥ 3	4/44 (91)	0.735	1.46	0.16 to	3/33 (85)	0.76	1.4	0.15 to

				13.0				13.8
PPH3								
< 13	2/35 (94)		1		2/35 (94)		1	
> 13	3/26 (89)	0.426	2.0	0.34 to 12.2	2/15 (87)	0.40	2.3	0.32 to 16.1
Ki67								
< 15	0/26 (100)		1		0/25 (100)		1	
≥ 15	5/34 (82)	0.040	-	-	4/24 (83)	0.014	*	*
Ki67								
< 30	1/38 (97)		1		1/37 (95)		1	
≥ 30	4/22 (82)	0.033	7.5	0.84 to 67.5	3/12 (75)	0.023	9.9	1.03 to 95.3
TILs								
Negative	4/55 (93)		1		3/45 (93)		1	
Positive	1/6 (83)	0.479	2.16	0.24 to 19.4	1/4 (75)	0.24	3.6	0.37 to 34.6
Luminal status ¶								
Luminal A	3/39 (92)		1		2/28 (93)		1	
Luminal B	2/22 (91)	0.847	1.2	0.20 to 7.41	2/22 (91)	0.777	1.33	0.19 to 9.42
Chemo therapy								
Yes	5/39 (87)		1		3/20 (85)		1	
No	0/22 (100)	0.089	0.22	0.024 to 1.95	1/30 (97)	0.15	0.22	0.023 to 2.10
Radiation therapy								
Yes	3/43 (93)		1		2/38 (95)		1	
No	2/17 (88)	0.499	1.84	0.33 to 11.0	2/12 (83)	0.19	3.9	0.48 to 24.1
Endocrine therapy								
Yes	5/39 (87)		1		4/36 (89)		1	
No	0/22 (100)	0.089	0.024	0 to 46.4	0/14 (100)	0.20	0.03	0 to 262
BMI-25 §								
< 25	1/31 (97)		1		1/26 (96)		1	
≥ 25	3/23 (87)	0.177	4.19	0.44 to 40.3	2/20 (90)	0.398	2.70	0.25 to 29.8
BMI-75p 								
< 75p	2/41 (95)		1		2/36 (94)		1	
≥ 75p	2/13 (85)	0.218	3.20	0.45 to 22.8	1/10 (90)	0.622	1.81	0.16 to 20.0
Smoking								
-Never smoked	3/15 (80)		1		2/12 (83)		1	
-Former smoker	0/23 (100)		0.003	Inf.	0/20 (100)		0.003	Inf.
-Ongoing smoking	0/14 (100)	0.020	0.003	Inf	0/12 (100)	0.052	0.003	Inf.

* HR (95% CI) was not computed as the equation did not converge no events occurred in one or more categories.

The histological grading is performed according to the Nottingham algorithm.

¶ Luminal A= ER+/HER2-/Ki67<15% & Luminal B=ER+/HER2-/Ki67≥15%.

- BMI-25 represents a dichotomized BMI < 25 or ≥ 25 on the BMI scale.

|| BMI-75p represents a dichotomized BMI with cut off < /≥ 75 percentile i.e. </≥ 26.8 on the BMI scale.

BMI, body mass index; HRT, hormonal replacement therapy; T, pathological tumor size in mm or category; LN, lymph node; N0, node negative; N+, node positive (assessed by pathologists); HER-2, human epidermal growth factor receptor 2; MAI, mitotic activity index; TILs, tumor infiltrating leucocytes; PPH3, phosphorylated phospho-histone 3.

Table 5. Changes in glucose and insulin related characteristics in the study groups

	Carbohydrate group (CH)				Fasting group (F)				Between groups		Normal range
	Admission values (A)	Preoperative Values (Pop)	Difference within group (A-Pop)	P-diff within group	Admission values (A)	Preoperative Values (Pop)	Difference within group (A-Pop)	P-diff within group	Difference between group CH vs F	P-diff between groups	
GLUCOSE (mmol/L)											4.0 to 6.0
Median	5.05	4.70			5.30	5.10					
Mean	5.37	5.22	- 0.15	0.625	5.34	5.11	- 0.23	0.009	+ 0.072	0.824	
IQR	4.88 to 5.05	4.25 to 5.50			4.80 to 5.80	4.70 to 5.40					
Range	4.40 to 10.00	3.2 to 12.1			4.20 to 6.40	3.30 to 6.90					
95%CI	4.90 to 5.80	4.48 to 5.96	- 0.79 to 0.49		5.15 to 5.53	4.89 to 5.33	- 0.39 to - 0.061		-0.59 to 0.73		
INSULIN (mIE/L)											< 29.1
Median	6.80	26.65			6.90	8.60					
Mean	9.43	33.68	+24.25	<0.0001	9.14	9.09	- 0.58	0.940	+ 24.31	<0.0001	
IQR	3.60 to 10.33	20.90 to 45.28			5.00 to 12.1	3.30 to 8.60					
Range	2.00 to 32.50	6.00 to 86.60			2.00 to 24.80	2.00 to 22.00					
95%CI	6.00 to 12.90	25.52 to 41.85	15.39 to 33.11		6.88 to 11.04	7.06 to 11.12	-1.58 to 1.47		15.34 to 33.27		
C-PEPTIDE (nM)											< 2.4
Median	0.61				0.66	0.68					
Mean	0.70	2.10	+1.40	< 0.0001	0.75	0.76	+0.004	0.926	+ 1.39	<0.0001	
IQR	0.50 to 0.83	1.50 to 2.41			0.53 to 0.66	0.53 to 0.68					
Range	0.34 to 1.91	0.71 to 5.10			0.38 to 1.92	0.40 to 1.45					
95%CI	0.57 to 0.83	1.67 to 2.53	0.98 to 1.83		0.64 to 0.86	0.66 to 0.85	- 0.083 to 0.091		1.03 to 1.77		
IGF-1 (nM)											5 to 28
Median	18.60	18.45			18.10	17.90					
Mean	18.67	18.98	+0.31	0.541	18.30	18.88	+0.58	0.145	+0.62	0.672	
IQR	14.2 to 23.0	14.15 to 18.45			15.70 to 21.60	18.80 to 23.30					
Range	8.5 to 30.6	10.20 to 33.50			8.60 to to 32.80	9.80 to 32.80					
95%CI	16.36 to 20.98	16.59 to 21.37	-0.72 to 1.35		16.34 to 20.25	16.89 to 20.86	- 0.21 to 1.36		-1.51 to 0.98		
IGFBP-3 (mg/L)											2.9 to 5.1
Median	4.55	4.05			4.20	4.40					
Mean	4.43	4.02	-0.42	< 0.0001	4.53	4.37	- 0.16	0.042	- 0.26	0.015	
IQR	3.95 to 5.05	3.50 to 4.58			4.00 to 5.30	3.80					
Range	3.00 to 5.60	2.70 to 5.20			2.80 to 6.50	2.80 to 5.60					
95%CI	4.11 to 4.75	3.74 to 4.30	-0.56 to - 0.27		4.24 to 4.83	4.11 to 4.64	-0.31 to - 0.0061		-0.46 to -0.051		

Figures

Figure 1

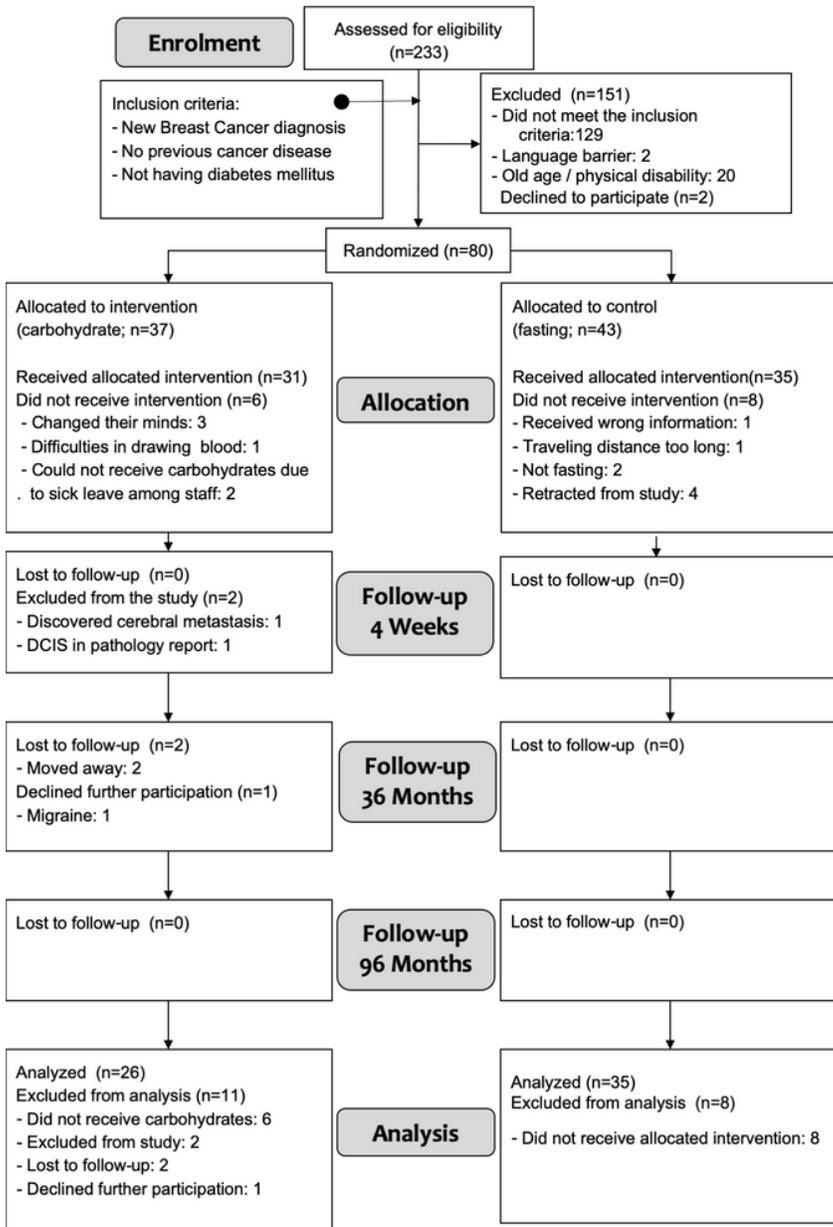


Figure 1

Study flow diagram

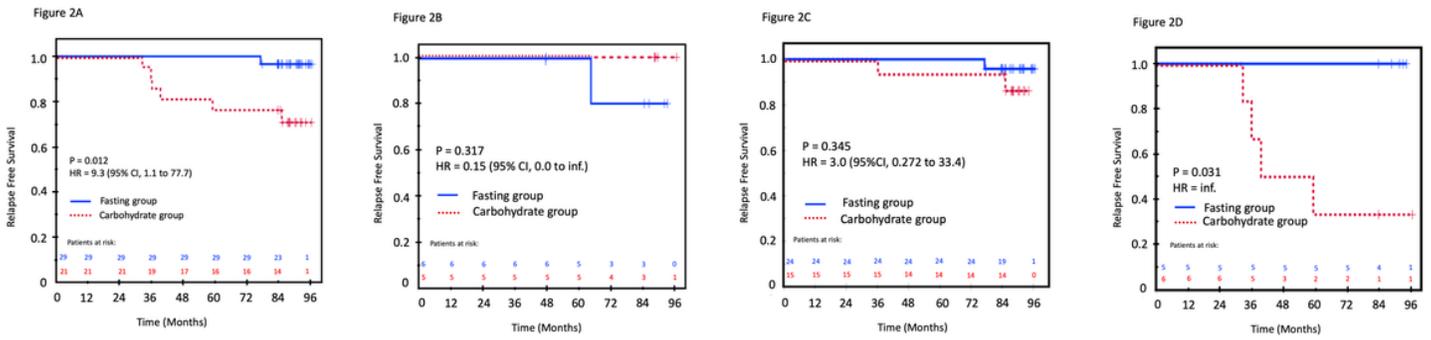


Figure 2

Relapse free Survival (RFS) in the Carbohydrate group and the Fasting group. Fasting group in blue solid line, Carbohydrate group red dotted line. Patients at risk above the X-axis with the same color coding of the treatment groups (blue=Fasting, and red = Carbohydrate group). Time in months on the X-axis and relative fraction of RFS on the Y axis. Censored patients are marked with a + sign on the survival curves. A. Relapse Free Survival in all ER-positive patients B. Relapse Free Survival in all ER-negative patients C. Relapse Free Survival in ER-positive, T1 patients D. Relapse Free Survival in ER-positive, T2 patients

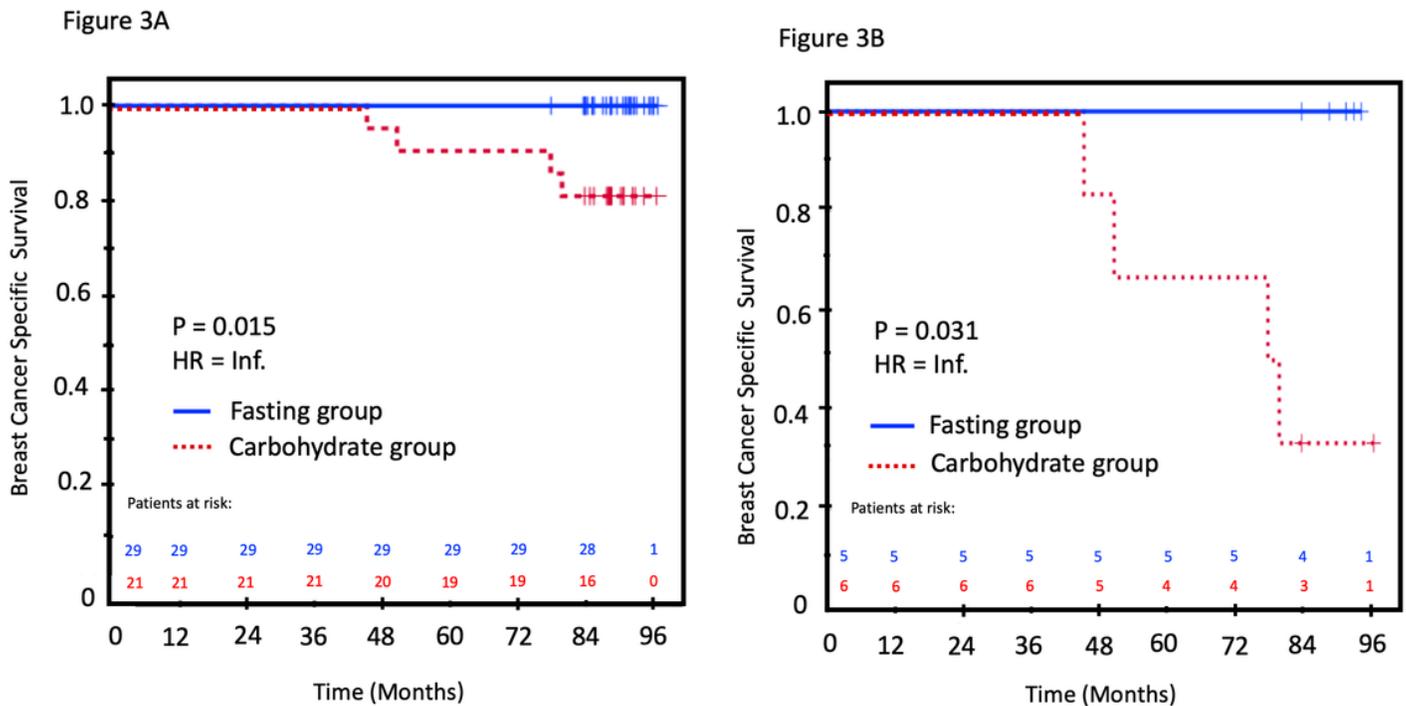


Figure 3

Breast Cancer Specific Survival (BCSS) in the intervention group and the control group. Fasting group blue solid line, Carbohydrate) group red dotted line. Patients at risk above the X-axis with the same color coding of the treatment groups (blue=fasting and red = carbohydrate group). Time in months on the X-axis and relative

fraction of RFS on the Y axis. Censored patients are marked with a + sign on the survival curves. A. BCSS in all ER-positive patients B. BCSS in ER-positive, T2 patients

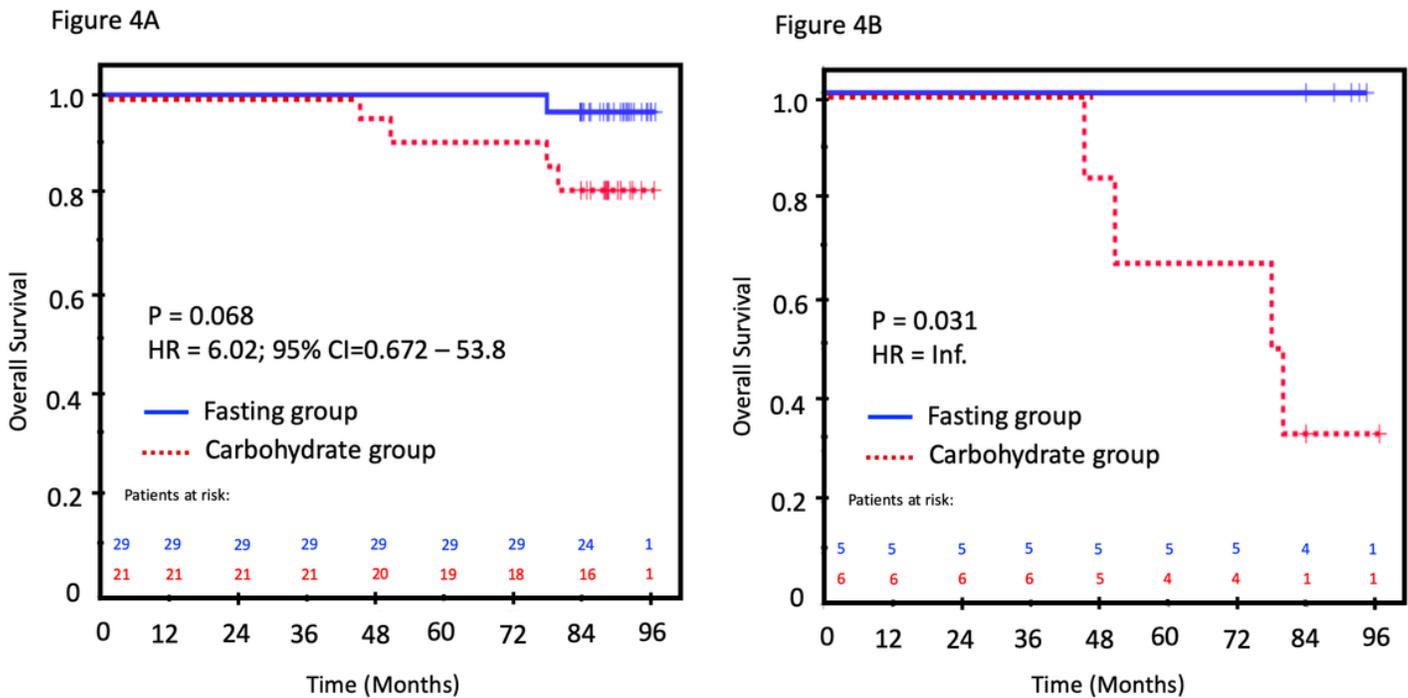


Figure 4

Overall Survival (OS) in the intervention group and the control group. Fasting group blue solid line, Carbohydrate) group red dotted line. Patients at risk above the X-axis with the same color coding of the treatment groups (blue=fasting and red = carbohydrate group). Time in months on the X-axis and relative fraction of RFS on the Y axis. Censored patients are marked with a + sign on the survival curves. A. OS in all ER-positive patients B. OS in ER-positive, T2 patients

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [CONSORT2010ChecklistLEndeetal2019toBMC.pdf](#)