



Universiteit
Leiden
The Netherlands

Short term fasting, IGF/insulin-axis and therapy outcome in patients with cancer

Groot, S. de

Citation

Groot, S. de. (2021, September 1). *Short term fasting, IGF/insulin-axis and therapy outcome in patients with cancer*. Retrieved from <https://hdl.handle.net/1887/3206649>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3206649>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/3206649> holds various files of this Leiden University dissertation.

Author: Groot, S. de

Title: Short term fasting, IGF/insulin-axis and therapy outcome in patients with cancer

Issue date: 2021-09-01

- 
- ¹ Department of Medical Oncology, Leiden University Medical Center, Leiden, The Netherlands
 - ² Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
 - ³ Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands
 - ⁴ Department of Medical Oncology, Medical center Leeuwarden, Leeuwarden, The Netherlands
 - ⁵ Department of Medical Oncology, Amphia, The Netherlands
 - ⁶ Department of Medical Oncology, Haga hospital, Den Haag, The Netherlands
 - ⁷ Department of Medical Oncology, Viecuri, The Netherlands
 - ⁸ Department of Medical Oncology, Deventer hospital, Deventer, The Netherlands
 - ⁹ Department of Medical Oncology, Noordwest hospital group, location Alkmaar, Alkmaar, The Netherlands
 - ¹⁰ Department of Medical Oncology, Hospital Gelderse vallei, The Netherlands
 - ¹¹ Department of Surgery, Leiden University Medical Center, The Netherlands
 - ¹² Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, The Netherlands
 - ¹³ Longevity Institute, School of Gerontology, and Department of Biological Sciences, University of Southern, United States
 - ¹⁴ IFOM FIRC Institute of Molecular Oncology, Milan Italy
 - ¹⁵ Department of Endocrinology, Leiden University Medical Center, The Netherlands
 - ¹⁶ BOOG Study Center, Amsterdam, The Netherlands

Chapter 4

Fasting mimicking diet as an adjunct to neoadjuvant chemotherapy for breast cancer in the multicentre randomized phase 2 DIRECT trial

Stefanie de Groot¹, Rieneke T. Lugtenberg¹, Danielle Cohen², Marij J.P. Welters¹, Ilina Ehsan¹, Maaïke P.G. Vreeswijk³, Vincent T.H.B.M. Smit², Hiltje de Graaf⁴, Joan B. Heijns⁵, Johanneke E.A. Portielje^{1,6}, Agnes J. van de Wouw⁷, Alex L.T. Imholz⁸, Lonneke W. Kessels⁸, Suzan Vrijaldenhoven⁹, Arnold Baars¹⁰, Elma Meershoek-Klein Kranenbarg¹¹, Marjolijn Duijm-de Carpentier¹¹, Hein Putter¹², Jacobus J.M. van der Hoeven¹, Johan W.R. Nortier¹, Valter D. Longo^{13,14}, Hanno Pijl¹⁵, Judith R. Kroep¹, Dutch Breast Cancer Research Group (BOOG)¹⁶

Nat Commun. 2020 Jun 23;11(1):3083. doi: 10.1038/s41467-020-16138-3.

This work was supported by grants from Pink Ribbon (2012.WO31.C155) and Amgen (20139098)

Abstract

Short-term fasting protects tumor-bearing mice against the toxic effects of chemotherapy while enhancing therapeutic efficacy. We randomized 131 patients with HER2-negative stage II/III breast cancer, without diabetes and a BMI over 19kg m^{-2} , to receive either a fasting mimicking diet (FMD) or their regular diet for 3 days prior to and during neoadjuvant chemotherapy. Here we show that there was no difference in toxicity between both groups, despite the fact that dexamethasone was omitted in the FMD group. A radiologically complete or partial response occurs more often in patients using the FMD (OR 3.168, $P=0.039$). Moreover, per-protocol analysis reveals that the Miller&Payne 4/5 pathological response, indicating 90-100% tumor-cell loss, is more likely to occur in patients using the FMD (OR 4.109, $P=0.016$). Also, the FMD significantly curtails chemotherapy-induced DNA damage in T-lymphocytes. These positive findings encourage further exploration of the benefits of fasting/FMD in cancer therapy.

Background

Extensive preclinical evidence suggests that short-term fasting and fasting mimicking diets (FMDs) can protect healthy cells against the perils of a wide variety of stressors, including chemotherapy, simultaneously rendering cancer cells more vulnerable to chemotherapy and other therapies¹⁻⁵. Essentially, fasting causes a switch in healthy cells from a proliferative state towards a maintenance and repair state. Malignant cells, in contrast, seem to be unable to enter this protective state because of oncoprotein activity, and therefore fail to adapt to nutrient scarce conditions. Instead, fasting deprives proliferating cancer cells of nutrients, growth and other factors, which renders them more sensitive to cancer therapy and increases cell death^{1,3}. The phenomenon by which normal but not cancer cells become protected to toxins is termed differential stress resistance (DSR)^{2,3} whereas the specific sensitization of cancer cells to stress is called Differential Stress Sensitization (DSS)^{1,6}.

Declines of plasma levels of insulin like growth factor-1 (IGF-1), insulin and glucose are among the mediators of the effects of fasting on cancer cells, as these factors can promote growth and prevent apoptosis^{1-4,6,7}. Fasting periods of at least 48 hours are required to induce a robust decrease in circulating glucose, IGF-1 and insulin levels^{6,8}. A very low calorie, low protein FMD was developed for its ability to cause metabolic effects on various starvation response markers similar to those caused by water-only fasting, while reducing the burden associated with a water only fast^{9,10}.

Small clinical studies showed that fasting as an adjunct to chemotherapy is safe and well tolerated, while it may reduce its toxicity¹¹⁻¹⁴. This multicentre, open label, randomized DIRECT study was designed to evaluate the impact of an FMD on toxicity as well as on the radiological and pathological response to chemotherapy for breast cancer.

Methods

Study design and patients

This is a randomized, controlled, observer-blind study. Eligible patients from 11 Dutch centers had histologically confirmed diagnosis of HER2-negative, stage II/III (cT1cN+ or ≥T2 any cN, cM0) early breast cancer, adequate bone marrow reserve (white blood counts $>3.0 \times 10^9/L$, absolute neutrophil count $\geq 1.5 \times 10^9/l$ and platelet count $\geq 100 \times 10^9/l$), adequate liver function (bilirubin $\leq 1.5 \times$ upper limit of normal (UNL) range, ALAT and/or ASAT $\leq 2.5 \times$ UNL, Alkaline Phosphatase $\leq 5 \times$ UNL), adequate renal function (calculated creatinine clearance ≥ 50 mL/min), normal cardiac function, a WHO performance state 0-2, age ≥ 18 years, BMI $> 19\text{kg m}^{-2}$, absence of diabetes mellitus, absence of allergies for FMD content, and signed informed consent. The

study (NCT02126449) was conducted in accordance with the Declaration of Helsinki (October 2013) and approved by the Ethics Committee of the Leiden University Medical Center in agreement with the Dutch law for medical research involving human subjects.

Drugs

Women received 8 cycles of neo-adjuvant AC-T chemotherapy (4 cycles doxorubicin 60 mg m⁻² and cyclophosphamide 600 mg m⁻² intravenously (i.v.)), followed by 4 cycles of T (docetaxel 100 mg m⁻² i.v.), or 6 cycles of neo-adjuvant FEC-T chemotherapy, consisting of 3 cycles of 5-fluorouracil, epirubicin and cyclophosphamide at a dose of 500, 100 and 500mg m⁻² i.v., respectively), followed by 3 cycles of T (docetaxel 100 mg m⁻² i.v.), all q 3 weeks. The anti-emetic agents granisetron (1 mg i.v.) or ondansetron (8 mg i.v.) were administered prior to chemotherapy. Dexamethasone (8mg i.v.) was administered shortly before chemotherapy for all cycles in the control group, whereas it was omitted during the AC or FEC courses in the FMD group, as dexamethasone may counteract the endocrine and metabolic effects of dietary intervention in the FMD group¹⁹.

Intervention

Women were randomized in a 1:1 ratio to receive the FMD (Xentigen™) or regular diet for 3 days prior to and on the day of each cycle of chemotherapy. The FMD is a 4-day plant-based low amino-acid substitution diet, consisting of soups, broths, liquids and tea (Supplementary figure 3). Calorie content declined from day 1 (~1200kcal), to days 2-4 (~200kcal). Moreover, the carbohydrates/proteins/fats energy ratio was approximately 3.5/1/2 on the first day, while complex carbohydrates were the main macronutrient (>80 energy%) the other subsequent 3 days. Patients were allowed to eat the diet components at any time of the designated day.

Randomization, masking and data storage

Patients were centrally randomized at the LUMC datacenter through block randomization with various block sizes stratified by stage (II versus III), estrogen receptor status (positive versus negative), BMI (<25kg m⁻² versus >25kg m⁻²) and chemotherapy regimen (AC-T versus FEC-T). The web based relational database management system ProMISe (<https://www.msbi.nl/promise/ProMISe.aspx>) was used for data storage and exchange.

Blood sampling

Venous blood samples were drawn prior to each chemotherapy administration (pre-chemotherapy on day -1 or day 0). Compliance with the diet was estimated by the following parameters: insulin, glucose, and IGF-1 (measured in a 9mL serum-separating tube). All samples were analyzed by the accredited clinical laboratories of the participating centers.

The effect of FMD on chemotherapy-induced DNA damage in peripheral blood mononuclear cells (PBMCs) was examined in a side study. Sodium heparinized venous blood samples (9mL) were collected for prior to the first cycle of chemotherapy and three hours after start of chemotherapy.

Toxicity and efficacy

The primary endpoint of the phase II and phase III parts of the study were grade III/IV toxicity and pathological complete response (pCR), respectively. Toxicity was documented by the physician and graded according to the Common Terminology Criteria for Adverse Events version 4.03 (CTCAE v.4.03). pCR was defined as the absence of residual invasive cancer within the breast and lymph nodes¹⁶, excluding isolated tumor cells (ITC).

Secondary endpoints included radiological response and pathological response according to the Miller and Payne (supplementary Table 1)¹⁶. Histopathology was centrally revised by one pathologist (DC), who was blinded to which treatment the patient received. Clinical response was measured by MRI or ultrasound of the breast halfway and at the end of therapy, according to RECIST1.1²⁰.

Quality of life (QoL)

Global health was assessed with the EORTC QLQ-C30²¹ before therapy (after randomization), halfway therapy, at the end of therapy and at six months follow-up. Higher scores (0-100 scale) on the functional scales indicate a better QoL.

Psychosocial distress was measured with the distress thermometer²², with an 11-point range from 0 (no distress) to 10 (extreme distress). Patients were asked to circle the number that best described the overall distress they experienced in the past week at 3 timepoints: halfway therapy, at the end of therapy and at six months follow-up.

DNA damage: isolation of PBMCs and γ -H2AX staining

PBMCs were isolated using Ficoll-Amidotrizoat (Pharmacy LUMC) gradient centrifugation according to the standard operating procedure of the Medical Oncology department of LUMC. Isolated PBMCs were carefully resuspended and 3 times washed in PBS (B. Braun, Melsungen, Germany). Samples were fixed in 1.5% formaldehyde and permeabilized in ice-cold pure methanol. Cells were washed 3 times in staining buffer (PBS with 5% bovine serum albumin (BSA, Sigma, St Louis, USA)) and stained for 30 minutes on ice with anti-CD45-PerCP-Cy5.5 (BD Bioscience, Breda, the Netherlands), clone 2D1 anti-CD3-PE (BD, clone SK7), anti-CD14-AF700 (BD, clone M5E2), anti-CD15-PE CF594 (BD, clone W6D3) and anti- γ -H2AX-AF488 (Biolegend, clone 2F3), followed by another washing step and resuspension in PBS. Per experiment we used

1,000,000 cells or more when available. The cell acquisition was performed immediately after the staining procedure on the flow cytometer (BD LSR Fortessa Flow Cytometer analyzer, BD Bioscience, Breda, The Netherlands) and data were analyzed using BD FACS Diva Software version 6.2. The CD45+ cells were gated, after which the CD3+ T-lymphocytes, CD3- non-T cells (also harboring B lymphocytes) or CD14+CD15-monocytes were analyzed for the geomean (as measure for the intensity) of γ -H2AX (Supplementary figure 2).

Statistical analysis

The primary endpoint of phase II of the study was grade III/IV toxicity. Based on trials with similar neo-adjuvant chemotherapy^{17,23,24}, the statistical power analysis revealed that a total number of 128 patients (64 patients in each arm) was required to be able to detect a 50% reduction of grade III/IV adverse effects with 80% power using a nominal significance level of 3.06% .

The primary endpoint of the phase III part of the study was pCR. We estimated the overall pCR rate to amount to 18%, based on studies examining similar third generation chemotherapy^{17,18,23}. Our sample size calculation revealed that we would require a total number of 212 patients (106 per treatment arm).

An interim analysis, focusing on feasibility and adverse events, was planned after completion of the phase II part of the protocol by 128 patients and was approved by the Ethics Committee of the Leiden University Medical Center. Early stopping rules included significantly more or unacceptable adverse events in either group. A data safety monitoring board conducted the interim analysis. Survival data will be reported after 5 years follow-up.

All parameters were tested for normality using a Kolmogorov-Smirnov test, with Bonferroni adjustment when evaluated in subgroups. Normally distributed parameters, if necessary after log transformation, were summarized as mean (and standard error of the mean (SEM)) and compared using independent or paired samples *t*-tests when appropriate. The non-normally distributed parameters were summarized as median (and 25th and 75th percentiles) and compared using a Mann-Whitney test for independent groups or Wilcoxon signed rank test for paired groups. The effect of FMD on efficacy of chemotherapy was analyzed using logistic regression, yielding univariate and multivariate odds ratios (ORs), 95% confidence intervals (CIs), and *P*-values. Multivariate analyses were adjusted for stratification factors²⁵. ER status, BMI, stage of disease and chemotherapy regimen. The Armitage's trend test was used to test an association between an ordinal variable and two categories. Mean changes in QoL from baseline to halfway, end of therapy and 6 months follow-up were assessed in linear

mixed models with 95% CIs. All tests were 2-tailed with a significance level of 0.05. All data were analyzed using IBM SPSS Statistics for Windows (Version 23.0. Armonk, NY: IBM Corp).

Results

Patient characteristics

From February 2014 to January 2018, 131 patients were randomized (see consort diagram, Figure 1). One patient withdrew informed consent before starting with chemotherapy and one patient was ineligible because of liver metastases, which were diagnosed a day after randomization. Of the 129 patients, 65 received FMD as an adjunct to chemotherapy and 64 patients used their regular diet. Thirty patients received FEC-T chemotherapy and 99 AC-T. Patient characteristics were equally distributed between groups (Table 1 and supplementary Table 2).

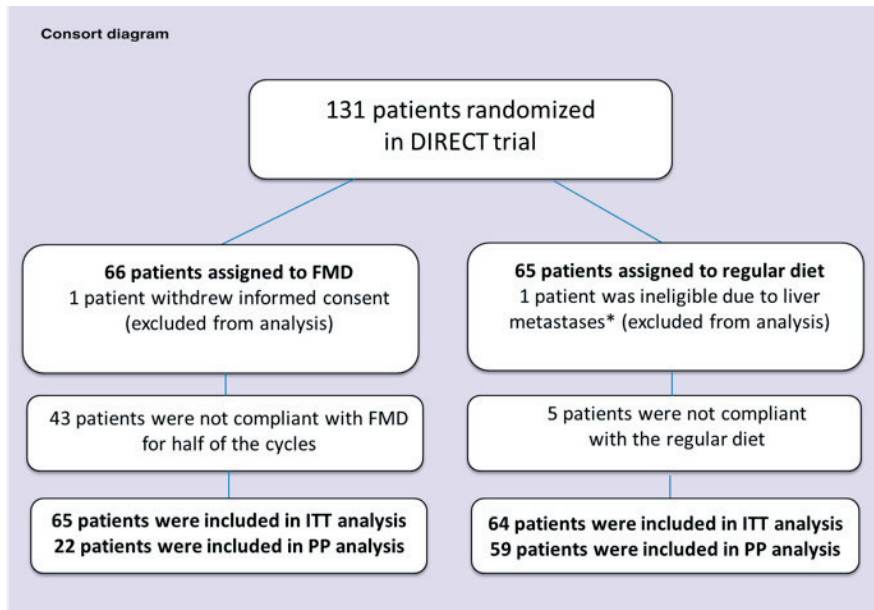


Figure 1: Consort diagram of the DIRECT study. This figure shows reasons for exclusion from the study and the numbers of patients included in the PP and ITT analyses. Abbreviations: FMD: fasting mimicking diet, ITT: Intention to treat, PP: Per protocol. * diagnosed the day after randomization.

Table 1: Patient characteristics.

	FMD (N=65)	Regular diet (N=64)
Median Age (range), Years	49.0 (31-71)	51.0 (27-71)
Median Body Mass Index (range), kg/m ²	25.7 (19.8-41.2)	26.0 (19.7-39.0)
WHO-status		
Grade 0	61 (93.8%)	60 (93.8%)
Grade 1	3 (4.6%)	4 (6.3%)
Unknown	1 (1.5%)	0 (0%)
Menopausal status		
Pre/Peri	27 (41.5%)	31 (48.4%)
Post	38 (58.5%)	31 (48.4%)
Unknown	0 (0%)	2 (3.1%)
T-classification		
T1	5 (7.7%)	6 (9.4%)
T2	42 (64.6%)	41 (64.1%)
T3	17 (26.2%)	15 (23.4%)
T4	1 (1.5%)	2 (3.1%)
N-classification		
N0	29 (44.6%)	33 (51.6%)
N1	28 (43.1%)	26 (40.6%)
N2	7 (10.8%)	4 (6.3%)
N3	1 (1.5%)	1 (1.6%)
Stage		
I (ineligible)	0 (0%)	1 (1.6%)
II	51 (78.5%)	48 (75.0%)
III	14 (21.5%)	15 (23.4%)
HR-status		
ER-/PR-	14 (21.5%)	7 (10.9%)
ER-/PR unknown	0 (0%)	1 (1.6%)
ER+/PR-	9 (13.8%)	9 (14.1%)
ER+/PR+	42 (64.6%)	47 (73.4%)
Chemotherapy regimen		
AC-T	52 (80.0%)	47 (73.4%)
FEC-T	13 (20.0%)	17 (26.6%)
Grade (BR)		
I	2 (3.1%)	2 (3.1%)
II	43 (66.2%)	42 (65.6%)
III	20 (30.8%)	19 (29.7%)
Unknown	0 (0%)	1 (1.6%)
Tumortype		
Ductal	53 (81.5%)	49 (76.6%)
Lobular	9 (13.8%)	13 (20.3%)
Other	3 (4.6%)	2 (3.1%)

Patient characteristics. Abbreviations: FMD: Fasting mimicking diet, HR: hormone receptor, AC-T: doxorubicin/cyclophosphamide followed by docetaxel, FEC-T: Fluorouracil/epirubicin/cyclophosphamide followed by docetaxel, BR: Bloom Richardson, ER: estrogen receptor, PR: progesterone receptor.

Interim analysis

Because the overall (both arms) pCR turned out to be significantly lower (11.7%) than anticipated (which would require the recruitment of twice as many participants to be able to detect the hypothesized pCR difference between both arms in a subsequent phase III study), in addition to the worse-than-expected compliance, the Data Safety Monitoring Board advised to dispense with the phase III study. Therefore, we here present the results of the phase II study.

Compliance

Fifty three out of 65 patients (81.5%) completed the first FMD cycle, whereas over 50% completed 2 FMD cycles, which could be sufficient to impact the tumor response to chemotherapy in view of the effects of only one or a few FMD cycles in enhancing the efficacy of chemotherapy in mice¹⁵. 22 out of 65 patients (33.8%) used the FMD for at least 4 cycles (all AC or FEC cycles), and 20.0% of the patients complied during all cycles of chemotherapy (supplementary Table 3). The main reason for non-adherence to the FMD was dislike of distinct components of the diet, perhaps induced by chemotherapy. In the regular diet group, 5 (7.8%) patients were not compliant (they decided to fast during one or more cycles of chemotherapy).

Intention to treat (ITT) analysis

Data on toxicity are shown in supplementary Table 4. Grade III/IV toxicity, scored during all cycles of chemotherapy, was not significantly different between the FMD group (75.4%) and the regular diet group (65.6%). No grade V toxicity occurred. The percentage of patients who discontinued chemotherapy did not significantly differ between groups (27.7% FMD *vs* 23.8% control, $P=0.580$). Notably, while side effects were similar in both arms, patients in the FMD arm did not receive dexamethasone before the AC chemotherapy cycles.

The radiological response and pathological response according to Miller and Payne are shown in Figure 2 and supplementary Table 5. The overall pCR rate was 11.7% and did not differ between the two groups (10.8% in FMD group versus 12.7% in control group; OR 0.830, 95% CI 0.282-2.442, $P=0.735$). Interestingly, the radiologically complete or partial response, as measured by MRI or ultrasound before surgery, occurred approximately 3 times more often in the FMD group compared to the control group in univariate (OR 2.886, 95% CI 1.012-8.227, $P=0.047$) and multivariate (OR 3.168, 95% CI 1.062-9.446, $P=0.039$) analyses. Accordingly, the proportion of patients with stable or progressive disease was 2.5 fold lower in the FMD group (11.3%) than in the control group (26.9%, Figure 2).

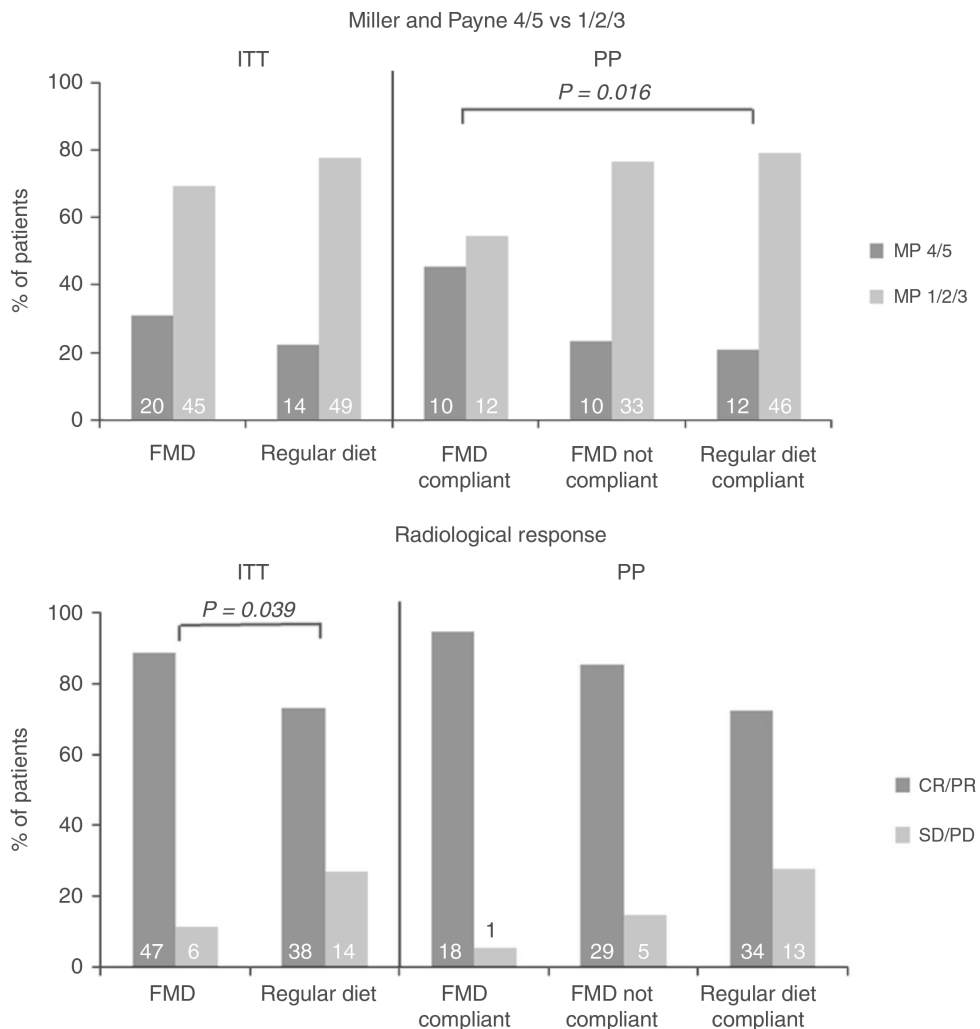


Figure 2: Tumor response data for the ITT and PP analysis. The pathological response was given for Miller and Payne pathological response score 4/5 (90-100% tumor cell loss) vs. 1/2/3 (less than 90% tumor cell loss). The radiological response was scored according RECIST1.1 and given for complete response + partial response vs. stable disease + progression disease. Abbreviations: FMD: fasting mimicking diet, ITT: Intention to treat, PP: Per protocol, MP: Miller and Payne, CR: complete response, PR: partial response, SD: stable disease, PD: progression disease. Logistic regression was used (2-sided).

The FMD affected various metabolic and endocrine parameters in the ITT analysis (Supplementary Table 6). At day -1/0 pre-chemotherapy, plasma insulin was significantly lower in the FMD group ($P=0.004$), while a trend for lower plasma glucose levels was observed in the FMD group ($P=0.062$). Urine ketone bodies were higher in the FMD group versus the control group ($P<0.0001$).

Data on global QoL and the distress thermometer are shown in Figure 3 and supplementary Figure 1, respectively. QoL was not significantly different between both groups in terms of global QoL ($P=0.841$) and overall distress ($P=0.674$).

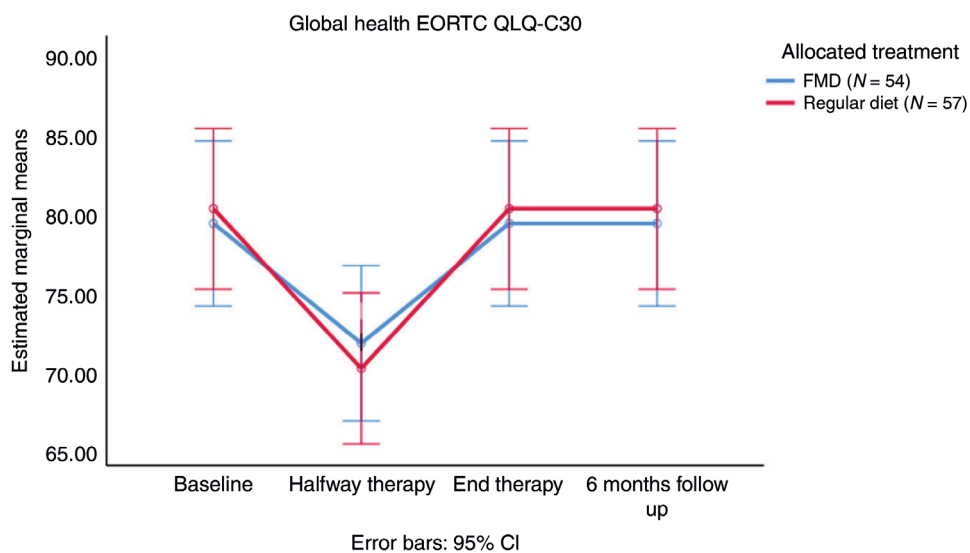


Figure 3: EORTC QLQ-C30 global health domain given for 4 timepoints: before therapy (after randomization), halfway therapy, at the end of therapy and at six months follow-up. Error bars indicate the 95% CI of the mean. Abbreviations: EORTC: European Organization for Research and Treatment of Cancer, FMD: fasting mimicking diet. CI: confidence interval. FMD N=57 and regular diet N=54. Armitage's trend test was used.



Per protocol (PP) analysis

A PP analysis was done to substantiate the effects of FMD on toxicity and efficacy of chemotherapy. Specifically, patients who were compliant with the FMD for at least half of the cycles were compared with those who were less compliant, and with the compliant control patients (i.e. the patients in the control group who did not fast on their own initiative).

Toxicity data of the PP analysis are shown in Table 2. Grade III/IV toxicity did not differ between FMD compliant patients (n=22) *vs.* control (n=59) group

Table 2: Grade III/IV toxicity in both groups (ITT) and in patients who were compliant with the FMD for at least half cycles of CT vs. control patients who did not fast on their own initiative (PP).

Grade III/IV	FMD (N=65)	FMD-C (N=22)	FMD-NC (N=43)	Control (N=64)	<i>P</i> -value (ITT)	<i>P</i> -value (PP)
Total	31 (47.7%)	11 (50.0%)	20 (46.5%)	36 (56.3%)	0.331	0.539
Neutropenic fever	5 (7.7%)	1 (4.5%)	4 (9.3%)	5 (7.8%)	0.980	0.548
Neutropenia	19 (29.2%)	6 (27.3%)	13 (30.2%)	18 (28.1%)	0.890	0.777

Grade III/IV side effects were scored according CTCAE4.03. Each side effect was scored maximal once per patient during the course. FMD; fasting mimicking diet, C: compliant, NC: Not compliant, ITT intention to treat, PP per protocol, CT: chemotherapy.

In the PP analysis, the pCR rate did not differ between the compliant FMD patients (13.6%) and controls (12.1%, OR 1.150, 95% CI 0.269-4.911, $P=0.850$, supplementary Table 5). However, the Miller and Payne pathological response 4/5 (90-100% tumor cell loss) occurred 3-4 times more often in patients using FMD in both univariate (OR 3.194, 95% CI 1.115-9.152, $P=0.031$) and multivariate analyses (OR 4.109, 95% CI 1.297-13.02, $P=0.016$, Figure 2) than in the control group. Furthermore, the more FMD cycles completed, the more patients had either a complete or partial radiological response to therapy (P for trend = 0.035, Figure 4). Both analyses were adjusted for hormone receptor status, TNM stage, BMI and chemotherapy regimen.

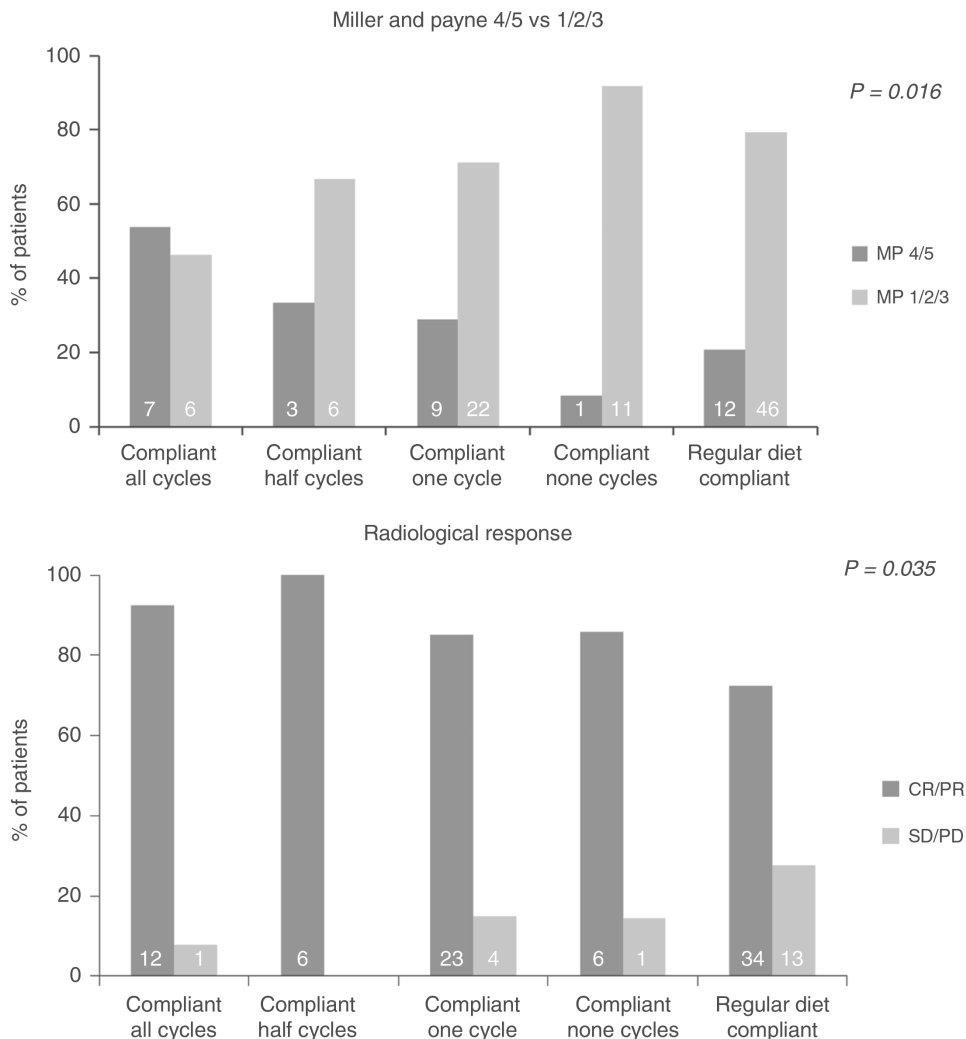


Figure 4: Tumor response data per number of cycles completed. The pathological response was given for Miller and Payne pathological response score 4/5 (90-100% tumor cell loss) vs. 1/2/3 (less than 90% tumor cell loss). The radiological response was scored according RECIST1.1 and given for complete response + partial response vs. stable disease + progression disease. P value is given for Armitage's trend test (2-sided). Abbreviations: FMD: fasting mimicking diet, MP: Miller and Payne, CR: complete response, PR: partial response, SD: stable disease, PD: progression disease.



In the PP analysis (Figure 5 and Supplementary Table 6), glucose was significantly lower in the compliant FMD group compared with the regular diet group before the first cycle and halfway therapy ($P=0.006$ and $P=0.042$, respectively). Insulin was significantly lower in the compliant FMD group compared with the control group before the first cycle and halfway therapy ($P=0.001$ and $P<0.001$, respectively). IGF-1 was significantly lower halfway therapy in patients who were compliant to the FMD in comparison to control patients ($P=0.025$). Ketone bodies were positive in most of the compliant FMD patients (93.3%) and rarely positive in the compliant control group (8.1%, $P<0.0001$).

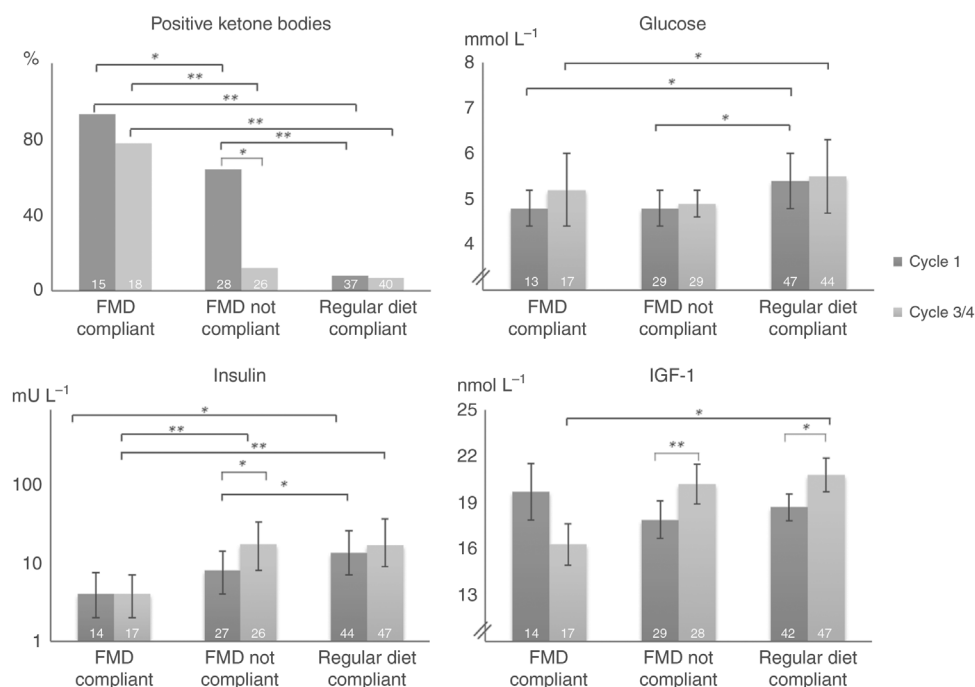


Figure 5: Metabolic and endocrine parameters before chemotherapy compared between compliant and non-compliant patients halfway therapy of the FMD group and the regular group. Values are measured on day -1 or day 0 before cycle 1 and halfway therapy. * P value < 0.05 , ** P value < 0.001 (2-sided). Error bars indicate the standard error of the mean (if data was normally distributed) or the 25% and 75% percentiles of the median (if data was non-normally distributed). Independent t-tests and Mann-Whitney tests were used. Reference values: glucose 3.1-6.4 mmol/L; insulin 0-20 mU/L; IGF-1 5.4-24.3 nmol/L. Abbreviations: FMD: fasting mimicking diet, IGF-1: Insulin-like growth factor 1.

The level of γ -H2AX intensity are reported in supplementary Table 7. Only compliant patients were included. γ -H2AX intensity increased 3 hours after chemotherapy in both groups for each cell type due to chemotherapy. The increase in DNA damage after chemotherapy was significantly less in CD45 + CD3+ T-lymphocytes from patients who had FMD as compared to patients using regular diet ($P=0.045$, Figure 6).

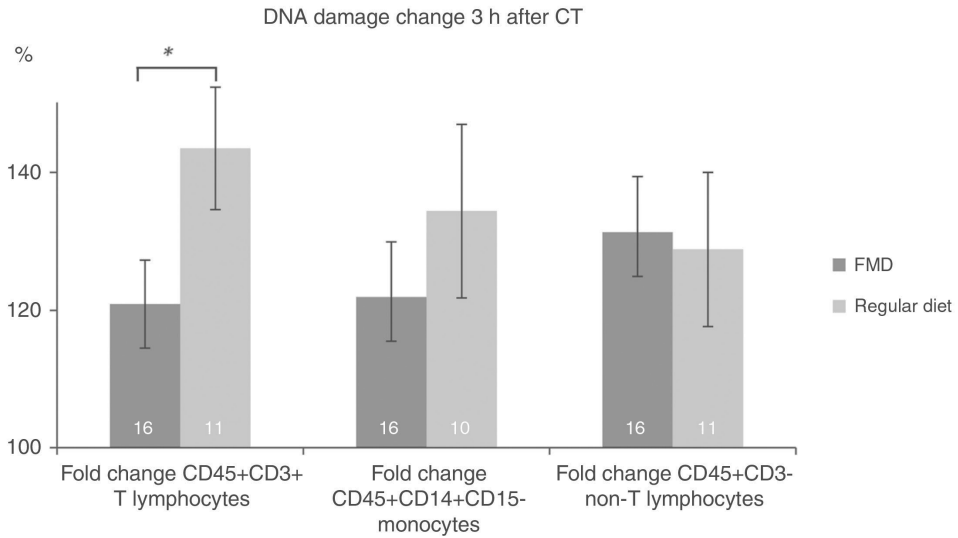


Figure 6: Difference of γ -H2AX intensity in CD45 + CD3+ lymphocytes of each patient before cycle 1 and 3 hours after chemotherapy, given as a percentage increase. Error bars indicate the standard error of the mean. Independent t-tests were used (2-sided). Abbreviations: FMD: fasting mimicking diet. * P=0.045.

Discussion

This is the first randomized controlled study evaluating the effects of an FMD on toxicity and efficacy of chemotherapy in patients with cancer. The results suggest that an FMD significantly reinforces the effects of neoadjuvant chemotherapy on the radiological and pathological tumor response in patients with HER2 negative early breast cancer. The ITT analysis reveals an increase of patients with a radiologically complete or partial response and a reduction of patients with radiologically stable/progressing disease in the FMD group compared to the control group. The PP analysis shows a beneficial effect of the FMD on the pathological response according to Miller and Payne. The more cycles of FMD were adhered to, the higher the percentage of Miller and Payne scores 4/5 (documenting > 90% tumor cell loss) in the surgical specimen (Fig. 4).

By chance, the percentage of patients with a triple negative tumor randomized to receive the FMD was double the percentage of those in the control group (Table 1). pCR is more likely to occur in case of triple negative tumors^{16, 17}. However, triple negative tumors were significantly less common in patients who complied with the FMD than in those who did not, while the response of the tumor to chemotherapy was clearly more favorable in compliant patients (Supplementary table 2). Moreover, the positive effects of the FMD persisted after adjustment for the receptor status of the tumor. These facts suggest that it was the FMD rather than the hormone receptor status which determined the better response of the tumor in agreement with the extensive pre-clinical data.

Patients using the FMD as an adjunct to chemotherapy did not experience more grade III/IV adverse events than patients who did not follow a diet, despite the fact that they were not prescribed dexamethasone in concert with FEC/AC. This suggests that the FMD may obviate the need for dexamethasone in the prevention of the side effects of chemotherapy. Importantly, DNA damage in T-lymphocytes was less in patients who received the FMD in combination with chemotherapy compared to those receiving chemotherapy while on a regular diet, suggesting that the FMD protected these cells against the induction of DNA damage by chemotherapy.

The study was meant to be a phase II/III study to evaluate the effects of the FMD on toxicity and efficacy of chemotherapy, respectively. However, a pre-defined interim analysis revealed a lower than anticipated overall pCR rate in the combined arms (albeit similar to the pCR rate in a similar trial of the same BOOG group¹⁸, necessitating the recruitment of twice as many participants to reliably judge the impact of the FMD on this primary outcome measure. Because this would prolong the study period and require additional funds, the DSMB advised to stop and report the results at the completion of phase II. Remarkably, the phase II study, involving only 131 patients, was sufficient to show benefits of the FMD in sensitizing breast cancer cells to chemotherapy, with efficacy demonstrated both at the clinical and pathology levels.

Pre-clinical data, that has been accumulating for over 10 years, indicates that fasting can protect cancer-bearing mice against the side effects of chemotherapy³, while sensitizing the tumor to its toxic effects^{1, 2}. Even one or a few cycles of FMD by itself can inhibit the progression of a wide variety of cancers and increase the therapeutic efficacy of chemotherapy in mice^{1, 15} but can also prime breast cancer and other tumor cell types to an attack by immune cells¹⁵. Accordingly, in spite of the fact that many patients could not adhere to the dietary regimen during all cycles of chemotherapy, our intention to treat analysis reveals benefits in terms of tumor response.

Only a few, generally small clinical studies have evaluated the potential of fasting to improve cancer treatment¹¹⁻¹⁴, primarily focusing on feasibility and toxicity of treatment. Just two of these trials were randomized¹¹⁻¹⁴, but the results were in line with those of the current study. Previously, we reported reduced hematological toxicity and DNA damage in circulating mononuclear cells in a small group of women who fasted for 24 hours prior to (neo)adjuvant chemotherapy for breast cancer¹¹. A second randomized study revealed improved QoL and less fatigue in breast- and ovarian cancer patients, who fasted for 60 hours around the time of chemotherapy¹⁴. Yet another study reported a trend towards less grade 3-4 neutropenia and reduced DNA damage in leucocytes in patients who fasted for 48-72 as compared to 24 hours around the time of platinum-based chemotherapy for a variety of malignancies¹². Finally, fasting for variable time

periods may reduce adverse events of chemotherapy, which was suggested by a case series of 10 patients with various cancer types¹³. These data agree with the current data, showing that the FMD is safe and effective as an adjunct to chemotherapy, at least in patients with a normal body mass index at inclusion.

Our data should be cautiously interpreted, particularly those of the PP analysis, which bears the risk of selection bias. However, the ITT analysis confirms the positive impact of the FMD on the radiological response, whilst clearly showing a trend in support of the PP positive effect on the pathological response. Moreover, due to self-selection bias patients in the control group decided to fast on their own initiative, which may have decreased the positive impact of the FMD in the ITT analysis.

In conclusion, the results of this study are the first to suggest that FMD cycles are safe and effective as an adjunct to chemotherapy in women with early breast cancer. These findings together with preclinical data encourage further exploration of the benefits of fasting/FMD in patients receiving a wide range of cancer therapies.

Acknowledgements

We are greatly indebted to the patients for participating in this study, and their physicians for including the patients: E. Göker (Alexander Monro Hospital), A.J.M. Pas (t Langeland Hospital) A.H. Honkoop (Isala).

References

1. Lee, C. *et al.* Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. *Sci Transl Med* **4**, 124ra127 (2012).
2. Di Biase, S. & Longo, V.D. Fasting-induced differential stress sensitization in cancer treatment. *Mol Cell Oncol* **3**, e1117701 (2016).
3. Raffaghello, L. *et al.* Starvation-dependent differential stress resistance protects normal but not cancer cells against high-dose chemotherapy. *Proc. Natl. Acad. Sci. U. S. A* **105**, 8215-8220 (2008).
4. Huisman, S.A. *et al.* Fasting protects against the side-effects of irinotecan treatment but does not abrogate anti-tumor activity in mice. *Br. J Pharmacol* (2015).
5. de Groot, S., Pijl, H., van der Hoeven, J.J.M. & Kroep, J.R. Effects of short-term fasting on cancer treatment. *J Exp Clin Cancer Res* **38**, 209 (2019).
6. Thissen, J.P., Ketelslegers, J.M. & Underwood, L.E. Nutritional regulation of the insulin-like growth factors. *Endocr. Rev* **15**, 80-101 (1994).
7. Pollak, M. Insulin, insulin-like growth factors and neoplasia. *Best Pract Res Clin Endocrinol Metab* **22**, 625-638 (2008).
8. Cahill, G., Jr., Felig, P., Owen, O. & Wahren, J. Metabolic adaptation to prolonged starvation in man. *Nord. Med* **83**, 89 (1970).
9. Brandhorst, S. *et al.* A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan. *Cell Metab* **22**, 86-99 (2015).
10. Nencioni, A., Caffa, I., Cortellino, S. & Longo, V.D. Fasting and cancer: molecular mechanisms and clinical application. *Nature Reviews Cancer* **18**, 707-719 (2018).
11. de Groot, S. *et al.* The effects of short-term fasting on tolerance to (neo) adjuvant chemotherapy in HER2-negative breast cancer patients: a randomized pilot study. *BMC Cancer* **15**, 652 (2015).
12. Dorff, T.B. *et al.* Safety and feasibility of fasting in combination with platinum-based chemotherapy. *BMC Cancer* **16**, 360 (2016).
13. Safdie, F.M. *et al.* Fasting and cancer treatment in humans: A case series report. *Aging (Albany, NY)* **1**, 988-1007 (2009).
14. Bauersfeld, S.P. *et al.* The effects of short-term fasting on quality of life and tolerance to chemotherapy in patients with breast and ovarian cancer: a randomized cross-over pilot study. *BMC Cancer* **18**, 476 (2018).
15. Di Biase, S. *et al.* Fasting-Mimicking Diet Reduces HO-1 to Promote T Cell-Mediated Tumor Cytotoxicity. *Cancer Cell* **30**, 136-146 (2016).
16. Ogston, K.N. *et al.* A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast* **12**, 320-327 (2003).
17. von Minckwitz, G. *et al.* Doxorubicin with cyclophosphamide followed by docetaxel every 21 days compared with doxorubicin and docetaxel every 14 days as preoperative treatment in operable breast cancer: the GEPARDUO study of the German Breast Group. *J Clin Oncol* **23**, 2676-2685 (2005).
18. Charehbili, A. *et al.* Addition of zoledronic acid to neoadjuvant chemotherapy does not enhance tumor response in patients with HER2-negative stage II/III breast cancer: the NEOZOTAC trial

- (BOOG 2010-01). *Ann Oncol* **25**, 998-1004 (2014).
19. Hickish, T. *et al.* Glucose intolerance during adjuvant chemotherapy for breast cancer. *J Natl Cancer Inst* **101**, 537 (2009).
 20. Eisenhauer, E.A. *et al.* New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* **45**, 228-247 (2009).
 21. Aaronson, N.K. *et al.* The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. *J Natl Cancer Inst* **85**, 365-376 (1993).
 22. Tuinman, M.A., Gazendam-Donofrio, S.M. & Hoekstra-Weebers, J.E. Screening and referral for psychosocial distress in oncologic practice: use of the Distress Thermometer. *Cancer* **113**, 870-878 (2008).
 23. Vriens, B.E. *et al.* Doxorubicin/cyclophosphamide with concurrent versus sequential docetaxel as neoadjuvant treatment in patients with breast cancer. *Eur J Cancer* **49**, 3102-3110 (2013).
 24. Martin, M. *et al.* Adjuvant docetaxel for node-positive breast cancer. *N Engl J Med* **352**, 2302-2313 (2005).
 25. Kahan, B.C. & Morris, T.P. Reporting and analysis of trials using stratified randomisation in leading medical journals: review and reanalysis. *BMJ* **345**, e5840 (2012).

Supplementary material

Supplementary Table 1: Miller-Payne criteria for grading pathological response after neoadjuvant chemotherapy.

MP Grade	Pathological characteristics of the primary tumor
1	No change or some alteration to individual malignant cells but no reduction in overall cellularity
2	A minor loss of tumor cells but overall cellularity still high; up to 30% loss.
3	Between an estimated 30% and 90% reduction in tumor cells.
4	A marked disappearance of tumor cells such that only small clusters or widely dispersed individual cells remain; more than 90% loss of tumor cells
5	No malignant cells identifiable in sections from the site of the tumor; only vascular fibroelastotic stroma remains often containing macrophages. However, ductal carcinoma in situ may be present

Supplementary Table 2: Patient characteristics of both groups, and of patients who were compliant with the FMD for at least half cycles of CT or those who were not and control patients who did not fast on their own Initiative.

	FMD (N=65)	FMD-C (N=22)	FMD-NC (N=43)	Regular diet (N=64)	Regular diet -C (N=59)
Median Age (range), Years	49.0 (31-71)	51.0 (44-69)	49.0 (31-71)	51.0 (27-71)	51.3 (27-71)
Median Body Mass Index (range), kg m ⁻²	25.7 (19.8-41.2)	25.9 (20.9-39.1)	25.4 (19.8-41.2)	26.0 (19.7-39.0)	26.3 (19.7-35.7)
WHO-status					
Grade 0	61 (93.8%)	20 (90.9%)	41 (95.3%)	60 (93.8%)	56 (94.9%)
Grade 1	3 (4.6%)	1 (4.5%)	2 (4.7%)	4 (6.3%)	3 (5.1%)
Unknown	1 (1.5%)	1 (4.5%)	0 (0.0%)	0 (0%)	0 (0%)
Menopausal status					
Pre/Peri	27 (41.5%)	11 (50.0%)	16 (37.2%)	31 (48.4%)	29 (49.2%)
Post	38 (58.5%)	11 (50.0%)	27 (62.8%)	31 (48.4%)	28 (47.5%)
Unknown	0 (0%)	0 (0%)	0 (0%)	2 (3.1%)	2 (33.9%)
T-classification					
T1	5 (7.7%)	1 (4.5%)	4 (9.3%)	6 (9.4%)	6 (10.2%)
T2	42 (64.6%)	16 (72.7%)	26 (60.5%)	41 (64.1%)	36 (61.0%)
T3	17 (26.2%)	4 (18.2%)	13 (30.2%)	15 (23.4%)	15 (25.4%)
T4	1 (1.5%)	1 (4.5%)	0 (0%)	2 (3.1%)	2 (3.4%)
N-classification					
N0	29 (44.6%)	8 (36.4%)	21 (48.8%)	33 (51.6%)	30 (50.8%)
N1	28 (43.1%)	10 (45.5%)	16 (41.9%)	26 (40.6%)	24 (40.7%)
N2	7 (10.8%)	3 (13.6%)	4 (9.3%)	4 (6.3%)	4 (6.8%)
N3	1 (1.5%)	1 (4.5%)	0 (0%)	1 (1.6%)	1 (1.7%)
Stage					
I (ineligible)	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)	1 (1.6%)
II	51 (78.5%)	16 (72.7%)	35 (81.4%)	48 (75.0%)	43 (75.0%)
III	14 (21.5%)	6 (27.3%)	8 (18.6%)	15 (23.4%)	15 (23.4%)
HR-status					
ER-/PR-	14 (21.5%)	3 (13.6%)	11 (25.6%)	7 (10.9%)	6 (10.1%)
ER-/PR unknown	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)	1 (1.7%)
ER+/PR-	9 (13.8%)	3 (13.6%)	6 (14.0%)	9 (14.1%)	9 (15.3%)
ER+/PR+	42 (64.6%)	16 (72.7%)	26 (60.5%)	47 (73.4%)	44 (74.6%)
Chemotherapy regimen					
AC-T	52 (80.0%)	18 (81.8%)	34 (79.1%)	47 (73.4%)	44 (74.6%)
FEC-T	13 (20.0%)	4 (18.2%)	9 (20.9%)	17 (26.6%)	15 (25.4%)

Grade (BR)					
I	2 (3.1%)	0 (0.0%)	2 (4.7%)	2 (3.1%)	2 (3.4%)
II	43 (66.2%)	15 (68.2%)	28 (65.1%)	42 (65.6%)	37 (62.7%)
III	20 (30.8%)	7 (31.8%)	13 (30.2%)	19 (29.7%)	19 (32.2%)
Unknown	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)	1 (1.7%)
Tumortype					
Ductal	53 (81.5%)	18 (81.8%)	35 (81.4%)	49 (76.6%)	44 (74.6%)
Lobular	9 (13.8%)	4 (18.2%)	5 (11.6%)	13 (20.3%)	13 (22.0%)
Other	3 (4.6%)	0 (0.0%)	3 (7.0%)	2 (3.1%)	2 (3.4%)

Patient characteristics. Abbreviations: CT: chemotherapy FMD: Fasting mimicking diet, C: compliant, NC: Not compliant, HR: hormone receptor, AC-T: doxorubicin/cyclophosphamide followed by docetaxel, FEC-T: Fluorouracil/epirubicin/cyclophosphamide followed by docetaxel, BR: Bloom Richardson, ER: estrogen receptor, PR: progesterone receptor.

Supplementary Table 3: Compliance in both arms.

	FMD (N=65)	Regular diet (N=64)
Compliant – all cycles and surgery		
Yes	10 (15.4%)	59 (92.2%)
No	55 (84.6%)	5 (7.8%)
Compliant – all cycles*		
Yes	13 (20.0%)	
No	52 (80.0%)	
Compliant – half of cycles**		
Yes	22 (33.8%)	
No	43 (66.2%)	
Compliant – first cycle		
Yes	53 (81.5%)	
No	11 (16.9%)	
Unknown	1 (1.5%)	
Reason for early stop FMD		
Taste	26 (51.0%)	
Nausea	10 (15.4%)	
Hunger	5 (9.8%)	
Stop chemotherapy	2 (3.9%)	
Other	8 (35.3%)	

Compliance given per group. * Compliance is defined as patients complied the FMD or regular diet all cycles of their treatment arm. ** Compliance is defined as patients complied to the FMD or regular diet half of the cycles of their treatment arm. Regular diet patients were not compliant if they were fasting for at least one cycle on their own. Abbreviations: FMD: Fasting mimicking diet.

Supplementary Table 4: Grade III/IV toxicity during 8 cycles of AC-T/FEC-T in both groups.

Grade III/IV	FMD (N=65)	Control (N=64)	P-value (ITT)
Total	49 (75.4%)	42 (65.6%)	0.224
Neutropenic fever	22 (33.8%)	12 (18.8%)	0.052
Neutropenia	33 (50.8%)	22 (34.4%)	0.060

Grade III/IV side effects were scored according CTCAE4.03. Each side effect was scored maximal once per patient during the course. FMD; fasting mimicking diet, C: compliance, ITT: intention to treat. Crosstabs were used.

Supplementary Table 5: Associations between tumor, patient characteristics, treatment arm and efficacy of chemotherapy.

Parameter		N	pCR	Univariate analysis	P value	Multivariate analysis	P value
BMI	< 25kg m-2	59	11 (18.6%)	1.000	0.032	1.000 0.308 (0.087-1.085)	0.067*
	≥ 25kg m-2	69	4 (5.8%)	0.269 (0.081-0.895)			
HR receptor status	ER +	106	8 (7.5%)	1.000	0.003	1.000 5.626 (1.624-19.48)	0.006*
	ER -	22	7 (31.8%)	5.717 (1.809-18.07)			
Stage	I/II	100	13 (13.0%)	1.000	0.402		
	III	28	2 (7.1%)	0.515 (0.109-2.430)			
CT schedule	AC-T	98	14 (14.3%)	1.000	0.136		
	FEC-T	30	1 (3.3%)	0.207 (0.026-1.643)			
ITT	FMD	65	7 (10.8%)	0.830 (0.282-2.442)	0.735	0.581 (0.174-1.942)	0.378
	Regular diet	63	8 (12.7%)	1.000			
PP	FMD C	22	3 (13.6%)	1.150 (0.269-4.911)	0.850	1.074 (0.216-5.344)	0.816
	FMD NC	43	4 (9.3%)	0.747 (0.204-2.734)			
	Regular diet C	58	7 (12.1%)	1.000			

Parameter		N	MP 4/5 vs. 1/2/3	Univariate analysis	P value	Multivariate analysis	P value
BMI	< 25kg m-2	59	22 (37.3%)	1.000	0.013	1.000 0.354 (0.152-0.825)	0.016*
	≥ 25kg m-2	69	12 (17.4%)	0.354 (0.157-1.801)			
HR receptor status	ER +	106	24 (22.6%)	1.000	0.032	1.000 2.437 (0.894-6.646)	0.082*
	ER -	22	10 (45.5%)	2.847 (1.096-7.396)			
Stage	I/II	100	30 (30.0%)	1.000	0.105		
	III	28	4 (14.3%)	0.389 (0.124-1.218)			
CT schedule	AC-T	98	26 (26.5%)	1.000	0.988		
	FEC-T	30	8 (26.7%)	1.007 (0.399-2.540)			
ITT	FMD	65	20 (30.8%)	1.556 (0.703-3.441)	0.275	1.526 (0.655-3.556)	0.328
	Regular diet	63	14 (22.2%)	1.000			
PP	FMD C	22	10 (45.5%)	3.194 (1.115-9.152)	0.031	4.109 (1.297-13.02)	0.016
	FMD NC	43	10 (23.3%)	1.162 (0.449-3.006)			
	Regular diet C	58	12 (20.7%)	1.000			

Parameter		N	Radiological response CR+PR vs. SD+PD	Univariate analysis	P value	Multivariate analysis	P value
BMI	< 25kg m-2	50	43 (86.0%)	1.000	0.214		
	≥ 25kg m-2	55	42 (76.4%)	0.526 (0.191-1.448)			
HR receptor status	ER +	83	67 (80.7%)	1.000	0.907		
	ER -	22	18 (81.8%)	1.075 (0.320-3.614)			
Stage	I/II	86	71 (82.6%)	1.000	0.376		
	III	19	14 (73.7%)	0.592 (0.185-1.893)			
CT schedule	AC-T	82	68 (82.9%)	1.000	0.334		
	FEC-T	23	17 (73.9%)	0.583 (0.195-1.742)			
ITT	FMD	53	47 (88.7%)	2.886 (1.012-8.227)	0.047	3.168 (1.062-9.446)	0.039
	Regular diet	52	38 (73.1%)	1.000			
PP	FMD C	19	18 (94.7%)	6.882 (0.832-56.91)	0.074	6.365 (0.759-53.37)	0.088
	FMD NC	34	29 (85.3%)	2.218 (0.706-6.963)			
	Regular diet C	47	34 (72.3%)	1.000			

Regression models are used to measure associations. The radiological response was scored according RECIST and given for complete response + partial response vs. stable disease + progression disease. Abbreviations: pCR: pathological complete response, BMI: body mass index, HR: hormone receptor status, FMD: fasting mimicking diet, C: Compliant, NC: Not compliant, ITT: Intention to treat, PP: Per protocol, MP: Miller and Payne, CR: complete response, PR: partial response, SD: stable disease, PD: progression disease. * Result from ITT multivariate analysis.

Supplementary Table 6: Metabolic and endocrine parameters first cycle.

Parameter	FMD (N=65)	FMD-C (N=53)	FMD-NC (N=11)	Regular diet (N=64)	Regular diet-C (N=59)	P-value (ITT)	P-value (PP)
Glucose (3.1-6.4mmol/L)	5.1	4.9	6.4	5.7	5.9	0.062	0.006
Insulin (0-20mU/L)	6.0	4.0	19.0	12.0	13.5	0.004	<0.0001
IGF-1 (5.4-24.3nmol/L)	17.4	17.3	20.0	17.9	18.0	0.925	0.602
IGF-BP3 (2.2-5.8mg/L)	3.7	3.7	3.8	4.0	4.0	0.601	0.570
CRP (<5.0mg/L)	3.0	3.5	3.0	2.0	2.0	0.007	0.010
Ketone bodies						<0.0001	<0.0001
Pos	32 (74.4%)	14 (93.3%)	10 (35.7%)	5 (12.5%)			
Neg	11 (25.6%)	1 (6.7%)	18 (64.3%)	35 (87.5%)			

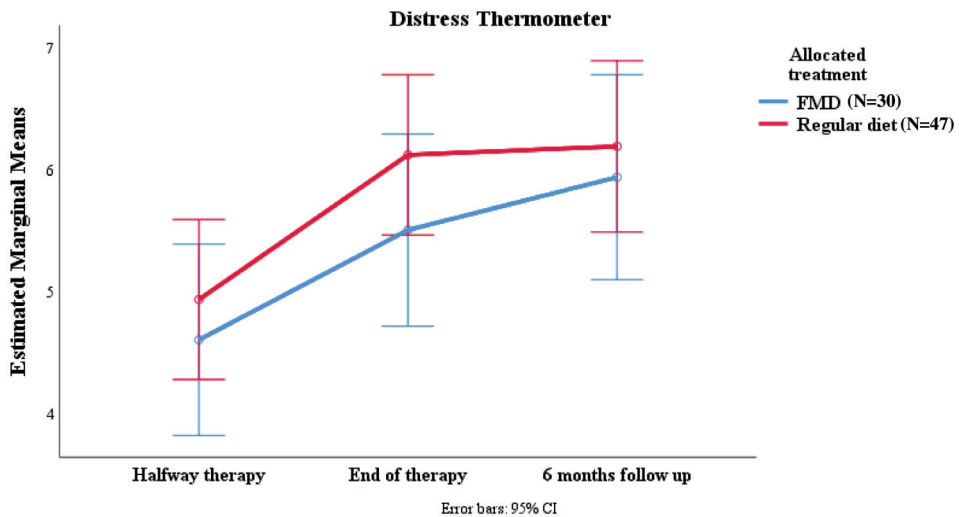
Insulin-like growth factor 1, IGF-BP3 insulin-like growth factor binding protein 3. ITT: intention to treat, PP: per protocol. Independent t-tests and Mann-Whitney tests were used.

Supplementary Table 7: γ -H2AX intensity in peripheral blood mononuclear cells.

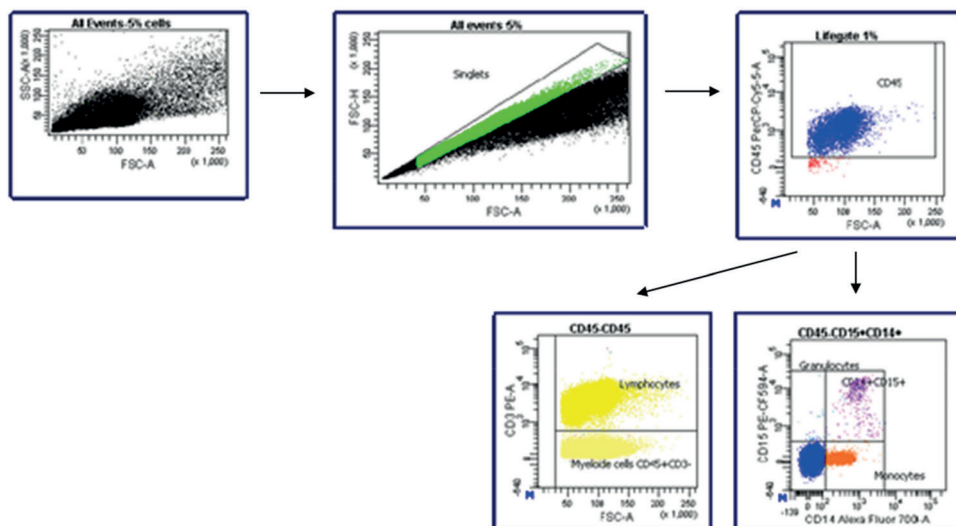
Parameter	N	Before CT (SE)	3 hours after CT (SE)	P value each group	P value between groups
CD45+CD3+ T-lymphocytes	FMD (n = 16)	143.4 (10.0)	173.4 (16.4)	0.011	0.045
	Regular diet (n = 11)	147.7 (8.6)	206.8 (14.6)	0.001	
CD45+CD14+CD15- monocytes	FMD (n = 16)	229.1 (16.0)	273.2 (21.5)	0.025	0.388
	Regular diet (n = 10)	230.7 (11.1)	309.5 (30.9)	0.021	
CD45+CD3- non-T cells	FMD (n = 16)	175.7 (10.6)	231.0 (21.3)	0.025	0.856
	Regular diet (n = 11)	185.6 (9.4)	237.8 (23.9)	0.037	

Paired comparison between pre- and 3 hours post- chemotherapy for different cell types in peripheral blood mononuclear cells. γ -H2AX intensity is given as mean. FMD; fasting mimicking diet. Independent and paired t-tests were used.





Supplementary figure 1: Psychosocial distress given for 3 timepoints: halfway therapy, at the end of the therapy and at six months follow-up. Error bars indicate the 95%CI of the mean. FMD: fasting mimicking diet. CI: confidence interval. FMD N=30 and regular diet N=47.



Supplementary figure 2: Gating strategy: The CD45+ cells were gated, after which the CD3+ T-lymphocytes, CD3- non-T cells (also harboring B lymphocytes) or CD14+CD15- monocytes were analyzed for the geomean (as measure for the intensity) of γ -H2AX.

Chemolieve™	Day 1	Day 2	Day 3	Day 4 *	Day 5	Day 6		
Morning	Tea	Energy Drink	Tea	Tea	TRANSITION DIET	Bar		
	Bar					NR-1 (2)**		
	Algal Oil (2)						Algal Oil (1)	
Lunch	Soup		Energy Drink	Soup		Soup		NORMAL DIET
	Chips							
	NR-1(2)							
Afternoon	Tea	Tea		Tea	Tea			
	Bar							
Dinner	Soup	Broth		Broth	Broth			
	Chips							

Supplementary figure 3: Chemolieve fasting mimicking diet schedule.

