

Supplementary Information:

Fasting mimicking diet as an adjunct to neoadjuvant chemotherapy for breast cancer: DIRECT
(BOOG 2013-04).

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

MP Grade	<i>PATHOLOGICAL CHARACTERISTICS OF THE PRIMARY TUMOR</i>
1	<i>No change or some alteration to individual malignant cells but no reduction in overall Cellularity</i>
2	<i>A minor loss of tumor cells but overall cellularity still high; up to 30% loss.</i>
3	<i>Between an estimated 30% and 90% reduction in tumor cells.</i>
4	<i>A marked disappearance of tumor cells such that only small clusters or widely dispersed individual cells remain; more than 90% loss of tumor cells</i>
5	<i>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.</i>

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 (3.9%)
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

<i>Parameter</i>		<i>N</i>	<i>pCR</i>	<i>Univariate analysis</i>	<i>P value</i>	<i>Multivariate analysis</i>	<i>P value</i>
BMI	< 25kg m-2	59	11 (18.6%)	1.000	0.032	1.000	0.067*
	≥ 25kg m-2	69	4 (5.8%)	0.269 (0.081-0.895)		0.308 (0.087-1.085)	
HR receptor status	ER +	106	8 (7.5%)	1.000	0.003	1.000	0.006*
	ER -	22	7 (31.8%)	5.717 (1.809-18.07)		5.626 (1.624-19.48)	
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		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)	0.660	0.478 (0.113-2.017)	0.315
	Regular diet C	58	7 (12.1%)	1.000		1.000	
<i>Parameter</i>		<i>N</i>	MP 4/5 vs. 1/2/3	<i>Univariate analysis</i>	<i>P value</i>	<i>Multivariate analysis</i>	<i>P value</i>
BMI	< 25kg m-2	59	22 (37.3%)	1.000	0.013	1.000	0.016*
	≥ 25kg m-2	69	12 (17.4%)	0.354 (0.157-1.801)		0.354 (0.152-0.825)	
HR receptor status	ER +	106	24 (22.6%)	1.000	0.032	1.000	0.082*
	ER -	22	10 (45.5%)	2.847 (1.096-7.396)		2.437 (0.894-6.646)	
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		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)	0.757	0.926 (0.328-2.614)	0.884
	Regular diet C	58	12 (20.7%)	1.000		1.000	
<i>Parameter</i>		<i>N</i>	Radiological response CR+PR vs. SD+PD	<i>Univariate analysis</i>	<i>P value</i>	<i>Multivariate analysis</i>	<i>P value</i>
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)			
Stage	I/II	83	67 (80.7%)	1.000	0.907		
	III	22	18 (81.8%)	1.075 (0.320-3.614)			
HR receptor status	ER +	86	71 (82.6%)	1.000	0.376		
	ER -	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		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)	0.172	2.715 (0.785-9.390)	0.115
	Regular diet C	47	34 (72.3%)	1.000		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							
Pos	32 (74.4%)	14 (93.3%)	10 (35.7%)	5 (12.5%)		<0.0001	
Neg	11 (25.6%)	1 (6.7%)	18 (64.3%)	35 (87.5%)			<0.0001

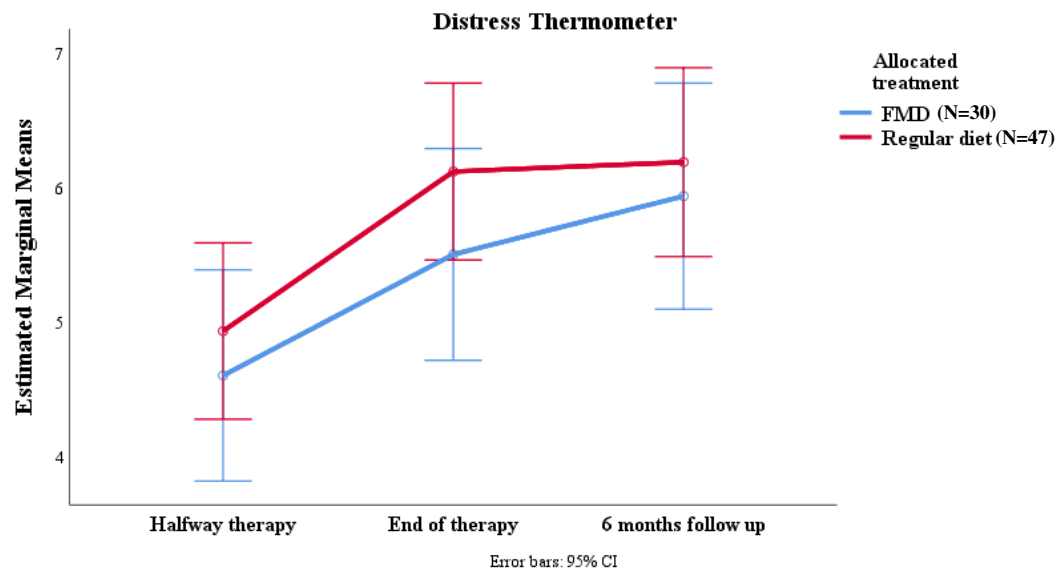
Insulin-like growth factor I, IGF-BP3 insulin- like growth factor binding protein 3. ITT: intention to treat, PP: per protocol. Independent t-tests and Mann-Whitney tests were used

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

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

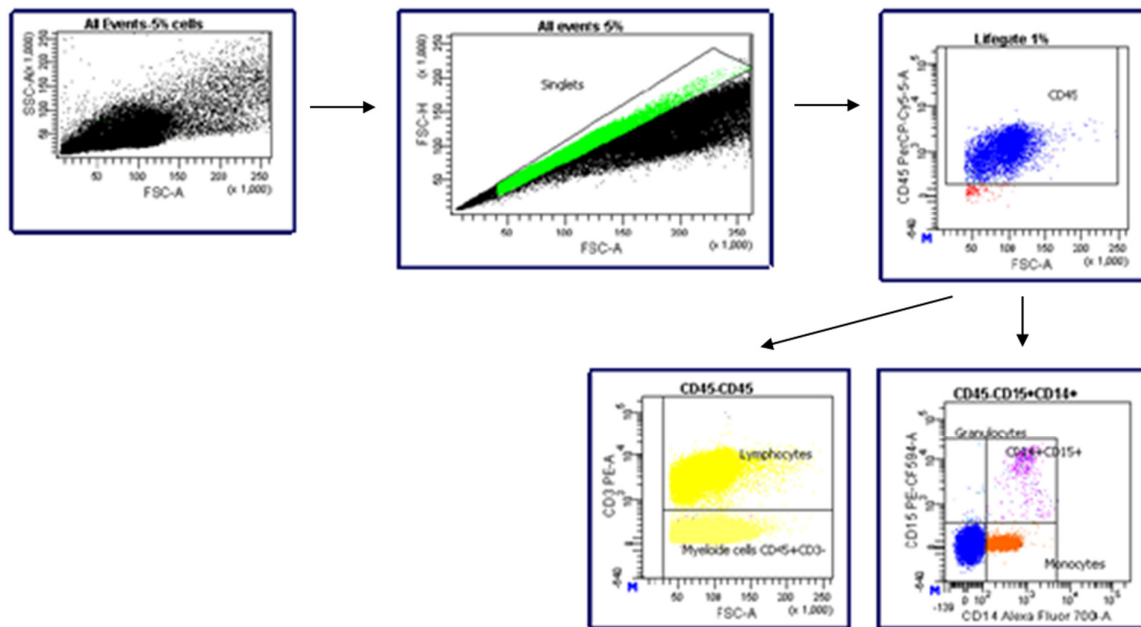
Paired comparison between pre- and 3 hours post- chemotherapy for different cell types in peripheral blood mononuclear cells. γ -H2AX intensity is given as mean. FMD; fasting mimicking diet. Independent and paired *t*-tests were used.

Supplementary figure 1.



Psychosocial distress given for 3 timepoints: halfway therapy, at the end of 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. Source data are provided as a Source Data file.

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.

Supplementary figure 3.

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

Chemolieve fasting mimicking diet schedule.