

Presence of early stage cancer does not impair the early protein metabolic response to major surgery

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Abstract

Background Combined bilateral mastectomy and reconstruction is a common major surgical procedure in women with breast cancer and in those with a family history of breast cancer. As this large surgical procedure induces muscle protein loss, a preserved anabolic response to nutrition is warranted for optimal recovery. It is unclear whether the presence of early stage cancer negatively affects the protein metabolic response to major surgery as this would mandate perioperative nutritional support.

Methods In nine women with early stage (Stage II) breast malignancy and nine healthy women with a genetic predisposition to breast cancer undergoing the same large surgical procedure, we examined whether surgery influences the catabolic response to overnight fasting and the anabolic response to nutrition differently. Prior to and within 24 h after combined bilateral mastectomy and reconstruction surgery, whole body protein synthesis and breakdown rates were assessed after overnight fasting and after meal intake by stable isotope methodology to enable the calculation of net protein catabolism in the post-absorptive state and net protein anabolic response to a meal.

Results Major surgery resulted in an up-regulation of post-absorptive protein synthesis and breakdown rates ($P < 0.001$) and lower net protein catabolism ($P < 0.05$) and was associated with insulin resistance and increased systemic inflammation ($P < 0.01$). Net anabolic response to the meal was reduced after surgery ($P < 0.05$) but higher in cancer ($P < 0.05$) indicative of a more preserved meal efficiency. The significant relationship between net protein anabolism and the amount of amino acids available in the circulation ($R^2 = 0.85$, $P < 0.001$) was independent of the presence of non-cachectic early stage breast cancer or surgery.

Conclusions The presence of early stage breast cancer does not enhance the normal catabolic response to major surgery or further attenuates the anabolic response to meal intake within 24 h after major surgery in patients with non-cachectic breast cancer. This indicates that the acute anabolic potential to conventional feeding is maintained in non-cachectic early stage breast cancer after major surgery.

Keywords Large breast (prophylactic) surgery; Non-cachectic Stage II breast cancer; Overnight catabolism; Anabolic response to feeding; Stable isotopes; Translational research

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Introduction

Combined bilateral mastectomy and reconstruction surgery is a major breast surgical procedure in patients with breast cancer and healthy women with a genetic predisposition to

breast cancer. A major surgical procedure is known to induce a systemic inflammatory response, increase the release of catabolic hormones, and is often associated with insulin resistance, perioperative starvation, and immobilization, all contributing more or less to postoperative weight and muscle

protein loss. Surgery in patients with weight-losing cancer leads to poor postoperative recovery, as reflected by increased complication rate and length of hospital stay and decreased survival.^{1,2} Disturbances in protein metabolism are observed in many cancer types, even when cachexia or weight loss is absent.^{3–5} To minimize the catabolic effects of a major surgical procedure in patients with cancer, insight is needed on whether disturbances are present in the normal protein metabolic response to surgery in these patients.

Previous animal research showed that healthy rats undergoing major surgery were able to mobilize protein from muscle and intestine in the postoperative period, whereas non-cachectic tumour-bearing rats were unable to do so.^{6,7} In line, non-cachectic tumour-bearing mice had less ability to respond to surgical trauma regarding muscle myofibrillar protein turnover.⁸ These data suggest that the presence of cancer negatively affects the metabolic response to surgery even in cancer types in which cachexia is not frequently present such as breast cancer.

Anabolic resistance to conventional medical food has been observed in patients with advanced cancer.^{9,10} The importance of optimizing the anabolic potential of nutrition prior to and after surgery in patients with cancer is illustrated by recent studies showing that the anabolic effect of perioperative nutrition in cancer was related to the patient's catabolic state before surgery.¹¹ Furthermore, the degree of muscle atrophy after surgery in patients with colorectal cancer was inversely related to the recovery of postprandial muscle protein synthesis after surgery.¹⁰ None of these studies, however, included a healthy control group. To minimize protein loss in patients with cancer undergoing surgery, detailed insight is needed in the acute catabolic effects of surgery and whether the anabolic response to feeding is abnormal in patients with cancer. This can only be studied when patients with cancer are compared with subjects undergoing an identical (large) surgical procedure and are completely healthy, but this latter group is difficult to find. Based on previous data, we hypothesize that the acute catabolic response to overnight fasting and the anabolic response to conventional medical food are suppressed in patients with breast cancer after surgery.

In the present study, we examined the acute protein metabolic response to major surgery in patients with early stage breast cancer and healthy controls with a genetic predisposition to breast cancer by studying protein and urea kinetics in the overnight-fasted state prior to and within 24 h after surgery by using innovative stable isotope techniques. In addition, we studied whether the acute anabolic response to conventional medical food was different between the patients with cancer and controls before and after surgery. Combined bilateral mastectomy and reconstruction surgery was selected as it is a relatively large and comparable surgical procedure in patients with breast cancer and in healthy women undergoing prophylactic surgery. Patients with Stage II breast cancer were selected, as weight loss, cachexia, and

anorexia, factors known to increase the complexity of the metabolic abnormalities, are often absent in these patients. The results from this study provide important insight on the independent and combined effects of surgery and cancer in protein metabolism and whether the acute anabolic potential to conventional feeding is altered in non-cachectic early stage breast cancer after major surgery.

Materials and methods

Study population

The study population consisted of women with Stage II breast malignancy and healthy control women with a genetic predisposition to breast cancer scheduled for breast surgery (*Tables 1* and Supporting information *Table S2*), recruited during visits at the Women's Oncology Clinic Winthrop Rockefeller Cancer Institute at the University Arkansas for Medical Sciences (UAMS), Little Rock, AR. Exclusion criteria were neoadjuvant therapy (e.g. radiotherapy, chemotherapy) and any surgery less than 4 weeks before the study, pre-existing cardiovascular, metabolic, or renal disease, or untreated metabolic diseases including liver or renal disease. Written informed consent was obtained from all subjects. The study was approved by the Institutional Review Board, UAMS, and the Protocol Review and Monitoring Committee of the Arkansas Cancer Research Center, UAMS, and therefore has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Breast surgery procedures

All subjects were scheduled for bilateral mastectomy with or without lymphadenectomy, which was immediately followed by reconstruction surgery unless this was not possible or declined by the subject. Patients with known breast cancer underwent intraoperative injection of unfiltered technetium 99 sulfur colloid in the subareolar plexus of the breast and lymphazurin blue in the upper inner volar surface of the arm for sentinel lymph node biopsy and axillary reverse mapping.¹² All patients underwent total skin sparing mastectomy (nipple skin sparing mastectomy) via a vertical incision from the limbus to the inframammary fold.¹³ Pectoralis fascia was taken in all patients. Initial reconstruction consisted of the placement of pectoralis submuscular tissue expanders or implants with a lateral sling accomplished with deantigenized cadaver dermis. The surgeries were carried out between 8:00 a.m. and 4:00 p.m. The bilateral mastectomy procedures were all performed by the same breast cancer surgeon (V.S.K.), whereas the reconstruction surgeries were performed by two plastic surgeons by using identical

Table 1 General characteristics and surgery details of the control and breast cancer groups

| | | Control group (n = 9) | Breast cancer group (n = 9) |
|------------------------------|--------------------|-----------------------|-----------------------------|
| Age | year | 49.4 ± 8.4 | 49.6 ± 10.4 |
| Weight | kg | 76.0 ± 20.0 | 77.6 ± 14.2 |
| Body mass index | kg/m ² | 28.2 ± 6.1 | 28.5 ± 5.1 |
| Fat-free mass index | kg/m ² | 16.5 ± 2.2 | 16.4 ± 1.9 |
| | % Normal | 103.2 ± 13.8 | 102.4 ± 11.9 |
| Fat-free mass extremities | kg | 18.7 ± 3.3 | 18.5 ± 3.3 |
| Fat mass index | kg/m ² | 7.9 ± 0.2 | 7.8 ± 0.2 |
| Fat mass | % Body weight | 40.7 ± 5.9 | 40.7 ± 6.7 |
| Fat mass trunk | kg | 16.2 ± 8.5 | 15.9 ± 5.2 |
| Handgrip strength | n | 187 ± 42 | 245 ± 59 |
| | n/kg fat-free mass | 4.2 ± 0.8 | 5.6 ± 1.6 |
| Handgrip endurance | % | 75 ± 9 | 72 ± 7 |
| Surgery details | | | |
| Duration total surgery | min | 442 ± 64 | 430 ± 163 |
| Blood loss | mL | 210 ± 163 | 343 ± 144 |
| Saline infusion | mL | 3116 ± 890 | 2600 ± 1001 |
| Haemoglobin pre-surgery | g/L | 13.7 ± 0.7 | 12.5 ± 1.3 |
| Haemoglobin post-surgery | g/L | 10.4 ± 1.1 | 10.3 ± 0.6 |
| Platelet count pre-op | k/μL | 248 ± 25 | 231 ± 52 |
| Platelet count post-op | k/μL | 191 ± 22 | 168 ± 44 |
| WBC lymphocytes pre-surgery | k/μL | 6.3 ± 3.1 | 6.9 ± 2.5 |
| WBC lymphocytes post-surgery | k/μL | 10.4 ± 2.3 | 10.4 ± 3.6 |

All values are means ± SEM. Data were analysed by unpaired Student's *t*-test. No significant differences from the control group were found.

WBC, white blood cell count.

techniques. General anaesthesia included opioid analgesics, and antibiotics, antiemetics, and laxatives were provided to all patients and healthy subjects intrasurgery and/or post-surgery. Disease and treatment history and surgical details were obtained from the medical chart.

Study protocol

All subjects were studied the day prior to surgery at the UAMS Clinical Center of the Translational Research Institute and the day after surgery at the Short Stay Unit of the UAMS Medical Center. Body weight, height, fat, and fat-free mass (FFM) were measured by dual-energy X-ray absorptiometry [Hologic QDR 4500/Version 12.7.3.1 (Bedford, MA)] and standardized for height¹⁴ to obtain BMI, FFM index, and FM index. Respiratory muscle function was assessed by measuring maximal expiratory pressure and inspiratory pressure by using a handheld mouth pressure device (micro respiratory pressure meter). Handgrip strength (viewed as a correlate of health in breast cancer survivors¹⁵) and endurance were assessed by Vernier Hand Dynamometry.^{5,16}

The metabolic part of the study days lasted 5.5 h (Supporting Information, Figure S1). Each study day started in the early morning after an overnight fast. One peripheral catheter was placed in an antecubital vein for stable isotope infusion and the other in a superficial dorsal vein of the hand or lower arm of the contralateral arm for blood sampling. The hand was placed in a thermostatically controlled hot box (internal temperature: 60°C) to mimic direct arterial sampling.¹⁷ A primed, constant, and continuous infusion of stable isotopes

was performed to measure their whole body rate of appearances (Supporting Information, Table S1).⁵ After 1.5 h of infusion, each subject ingested within 5 min a commercial medical food often used in cancer care (Boost High Protein drink (8 fl oz, 240 calories, 15 g protein, 6 g fat, 33 g carbohydrates) to which L-[¹⁵N]-phenylalanine (158.8 mg) was added to study first-pass splanchnic amino acid extraction.⁵

Biochemical analysis

Blood was processed, stored at -80°C, and analysed batchwise. Stable isotope enrichments and plasma amino acid concentrations were analysed by liquid chromatography-tandem mass spectrometry by isotope dilution.¹⁸ Plasma C-reactive protein (CRP) levels were measured by using sandwich enzyme-linked immunosorbent assay (HS-ELISA), insulin by radioimmunoassay (Insulin RIA kit; Linco Research Inc., St. Charles, MO), and glucose by COBAS-FARA semiautomatic analyser (Uni Hit III, 07367204 concentration, Roche, Basel, Switzerland). Homeostasis model assessment (HOMA) score, a marker of insulin resistance, was subsequently calculated.¹⁹

Calculations

Whole body protein synthesis, protein breakdown, urea synthesis, and splanchnic extraction of dietary phenylalanine as an analogue for dietary amino acids were calculated from the stable isotope enrichments in the overnight-fasted

(post-absorptive) and postprandial state,^{18,20} see Appendix. Post-absorptive net protein catabolism was calculated as the difference between protein breakdown and synthesis rates and net protein anabolic response to the meal as the difference in protein synthesis and breakdown rates. Plasma amino acid concentrations were analysed to obtain information about the availability of the dietary amino acids in the systemic circulation for net protein anabolism.

Statistical analysis

Results are expressed as mean \pm standard error (SE). If data failed the normality or equal variance test, data were log-transformed. Unpaired Student's *t*-test was used to determine differences in general characteristics between the groups. The median value of protein synthesis, breakdown, and net protein catabolism (=protein breakdown – synthesis) at 70, 80, and 90 min was used as the post-absorptive value as a steady state was obtained for the tracer–tracee ratios of PHE5, TYR4, and TYR2 (Supporting Information, Figure S3). The 3.5 h integral (nmol/kg FFM/3.5 h) after intake of the meal was used as the postprandial value of protein synthesis, breakdown, and net protein anabolism (=protein synthesis – breakdown). UREA1 tracer–tracee ratio kinetics (Supporting Information, Figure S3) revealed no steady state in the post-absorptive state post-surgery. Therefore, the curves were fitted by using a mono-exponential model in the post-absorptive (*t*: 0–90 min) and in the postprandial state (*t*: 90–300 min). Whole body urea synthesis in the post-absorptive state and the change in urea synthesis after meal intake (difference between postprandial and post-absorptive urea synthesis) were subsequently calculated pre-surgery and post-surgery.

Two-way repeated-measures analysis of variance (ANOVA) (general linear model) was carried out with group (cancer vs. control) and surgery (pre-surgery vs. post-surgery) as factors. Bonferroni post hoc test was applied when significant interactions were observed. The relation between net protein anabolism vs. postprandial amino acid appearance in the systemic circulation after splanchnic extraction was analysed with two-tailed tests of significance by using Pearson's correlation coefficients and linear regression analysis. The level of significance was set at $P < 0.05$. GRAPHPAD PRISM (Version 6.07) and SPSS (version 20) were used for curve fitting and data analysis. The level of significance was set at $P < 0.05$.

Results

Patient characteristics

We approached for this study 32 eligible subjects, and 20 of them consented. Nine patients (Stage II) and nine

controls completed the study (i.v. malfunctioned in two subjects) (Table 1). Age, body weight and composition, and muscle function were not different between the groups prior to surgery. In the year prior to enrolment, five patients (56%) underwent chemotherapy (last chemo session > 40 days prior to study enrolment), and three patients with cancer were undergoing hormonal therapy at the time of study enrolment (Supporting Information, Table S2). Five of the healthy control subjects (56%) had undergone hysterectomy (or combined with salpingo-oophorectomy) as part of the prophylactic approach 1 year preceding surgery. Besides bilateral mastectomy, seven out of the nine subjects with cancer and all nine healthy control subjects underwent reconstruction surgery (Supporting Information, Table S2). The total duration of surgery, blood loss, plasma haemoglobin, platelet, and white blood cell counts after surgery were comparable between the groups (Table 1). Surgery resulted in higher values for plasma CRP ($P < 0.001$), glucose, and HOMA index ($P < 0.01$) in both groups (Supporting Information, Figure S2).

Whole body protein metabolism in the breast cancer and healthy groups and the effects of surgery

Whole body post-absorptive protein breakdown and synthesis rates were not different between the two groups prior to surgery (Table 2). Surgery resulted in higher values for protein breakdown ($P < 0.01$) and synthesis ($P < 0.001$) and in lower net protein catabolism ($P < 0.01$), urea synthesis ($P < 0.05$), and plasma sum of all measured branched-chain acid concentration ($P < 0.001$) and sum of all amino acid concentration ($P < 0.01$). Furthermore, plasma urea concentration tended to be lower after surgery ($P = 0.06$).

Figure 1 shows the changes in postprandial kinetics in time of amino acid appearance in the systemic circulation after meal intake, as well as the protein anabolic response and plasma sum of all amino acid concentrations. The peak in amino acid appearance in the systemic circulation and net protein anabolism was reached preoperatively at 60 min after meal intake in both groups, but normalization towards baseline values was not obtained even at 3.5 h. After surgery, the peaks were lower, and a plateau was obtained. Plasma sum of all measured amino acid concentrations after intake was higher values in the cancer group.

Surgery resulted in higher values for protein synthesis ($P < 0.01$) and breakdown ($P < 0.001$) throughout the study day (Supporting Information, Figure S4). Furthermore, surgery resulted in a reduced net protein anabolism ($P < 0.05$) (Figures 1 and 2), which was associated with an increased splanchnic extraction of dietary amino acids ($P < 0.001$) (Figure 2). The meal-induced changes in protein metabolism (difference between postprandial and

Table 2 Post-absorptive whole body protein and urea kinetics and plasma amino acid and urea concentrations before and after surgery in the control and breast cancer groups

| | Control group (n = 9) | | Breast cancer group (n = 9) | | |
|--|-----------------------|---------------|-----------------------------|--------------|-------------|
| | Pre-surgery | Post-surgery | Pre-surgery | Post-surgery | |
| Protein breakdown | 64.4 ± 2.3 | 76.7 ± 3.1 | 66.8 ± 2.5 | 74.7 ± 3.8 | S:** |
| Protein synthesis | 59.1 ± 2.2 | 72.5 ± 3.2 | 61.6 ± 2.2 | 70.2 ± 3.4 | S:*** |
| Net protein catabolism | 5.3 ± 0.3 | 4.2 ± 0.3 | 5.1 ± 0.5 | 4.4 ± 0.3 | S:** |
| Urea synthesis | 756.9 ± 74.4 | 656.8 ± 100.5 | 723.1 ± 96.6 | 532.4 ± 63.3 | S: * |
| Plasma amino acid and urea concentration | | | | | |
| Sum of all branched-chain amino acids | 304 ± 13 | 222 ± 1 | 278 ± 12 | 219 ± 14 | S:*** |
| Sum of all measured amino acids | 2530 ± 56 | 2238 ± 132 | 2276 ± 65 | 1962 ± 115 | S:**, G:* |
| Urea | 4.5 ± 0.5 | 3.5 ± 0.5 | 3.7 ± 0.3 | 3.4 ± 0.5 | S: P = 0.06 |

Protein synthesis, breakdown, net breakdown, and urea synthesis are in $\mu\text{mol/kg FFM/h}$ (means \pm SEM). Concentration of the sum BCAA and all measured amino acids are μM , and mM for urea. Two-factor repeated-measures ANOVA was used to test surgery (S) and group (G) effect.

FFM, fat-free mass.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$; there was no surgery \times group interaction.

post-absorptive values) are presented in *Figure 2*. Surgery resulted in lower values for meal-induced increase in protein synthesis ($P < 0.05$) without significantly influencing protein breakdown, resulting in lower values for net protein anabolism ($P < 0.01$). Net protein anabolism was higher in the cancer group ($P < 0.05$) (*Figures 1 and 2*), which was associated with a tendency towards higher meal-induced increase in protein synthesis ($P = 0.08$) (*Figure 2*). No group \times surgery interaction effects were observed. A highly significant relationship was found between net protein anabolism and postprandial amino acid appearance in the systemic circulation after meal intake ($R^2 = 0.85$, $P < 0.001$) (*Figure 3*), which remained after stratification for group and surgery (pre-surgery vs. post-surgery). No cancer or surgery effect was observed in the meal-induced change in whole body urea synthesis (*Figure 4*).

Discussion

Major surgery results in reduced overnight net protein catabolism and lower anabolic response to food intake within 24 h post-surgery. The presence of non-cachectic early stage breast cancer does not impair the early protein metabolic response to surgery.

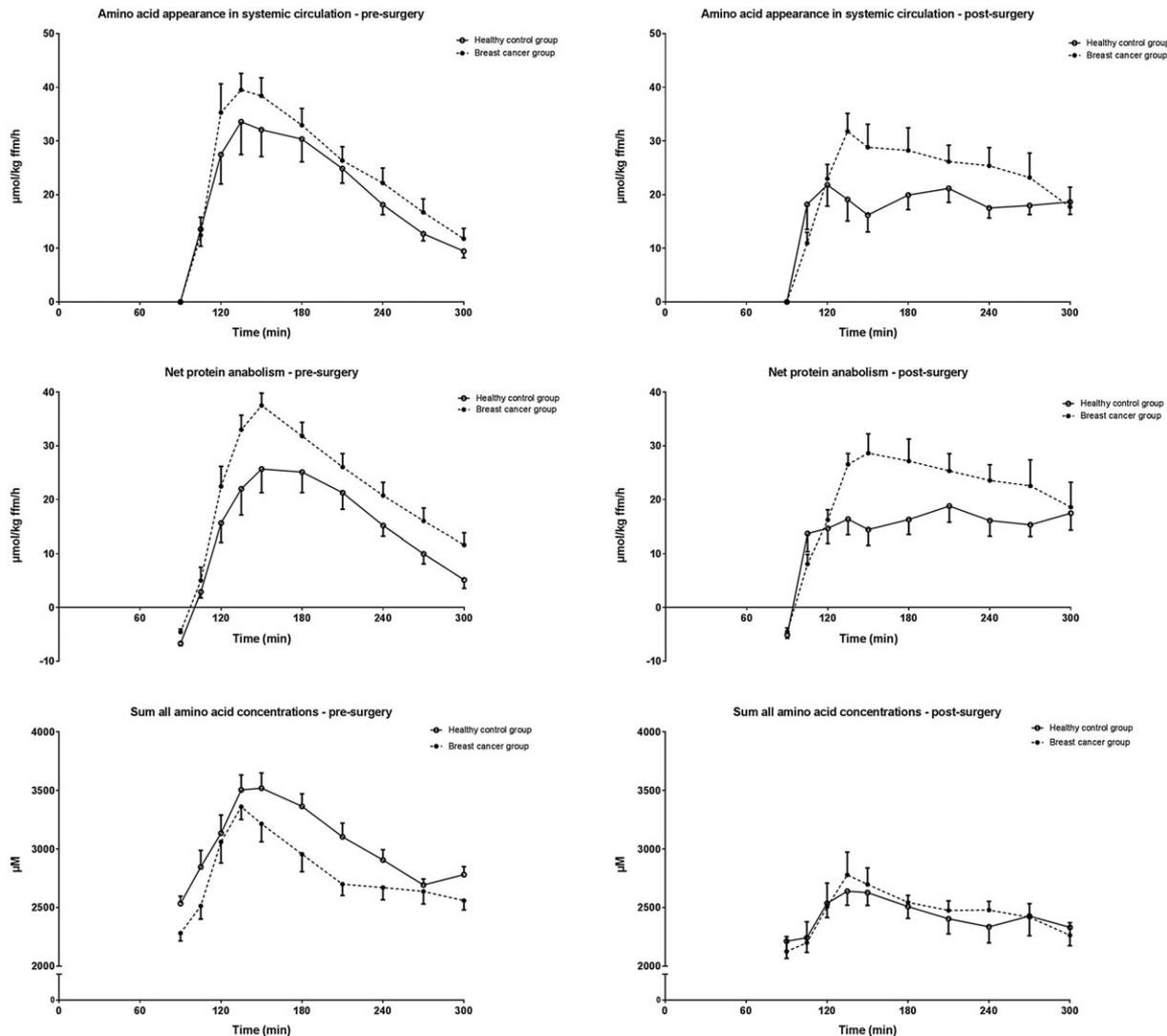
We studied patients with breast cancer in a stable period of their disease with preserved values for nutritional status and muscle function. We recruited patients with Stage II (non-metastatic) cancer without evidence of cachexia or recent involuntary weight loss and without an enhanced basal systemic inflammatory response. Therefore, we were able to directly study the effect of the presence of cancer on the metabolic response to breast surgery without interference of additional metabolic abnormalities induced by cachexia. These patients with non-cachectic weight-stable

early stage breast cancer were characterized by lower plasma amino acid concentrations in line with previous data in breast cancer²¹ but unchanged post-absorptive protein kinetics, indicating increased clearance (disposal) of amino acids.²²

Metabolic effects of breast surgery

The metabolic effects of breast surgery were studied as this type of surgery is known to cause more cancer-related fatigue and more disruption in activity levels than other less invasive surgery procedures.²³ Breast surgery was a major invasive surgical procedure for all our subjects as reflected by the duration of surgery (~7 h), total blood loss, and changes in clinical laboratory values. We found that breast surgery increased both whole body protein breakdown and synthesis independent of the presence of cancer. Many factors might contribute to the up-regulated protein turnover post-surgery, i.e. the observed enhanced systemic inflammatory response and insulin resistance (elevated HOMA index). Enhanced amino acid transfer is needed after major surgery for energy, synthesis of plasma proteins (i.e. the acute phase proteins), protein synthesis for wound healing, and to supply nutrients for the immune system. In the early phase of recovery, mobilization of proteins from pools with a rapid turnover such as gut and liver (visceral protein breakdown) will occur, whereas in a later phase, proteins from slower turnover pools (i.e. muscle) are mobilized.²⁴ Previously, decreased,²⁵ increased,²⁶ and unchanged²⁷ protein breakdown rates were observed after surgery, likely due to variations in the extent of trauma, postoperative phase (early or late), and methods used to quantify protein breakdown. A previous study found increased albumin synthesis levels after major abdominal

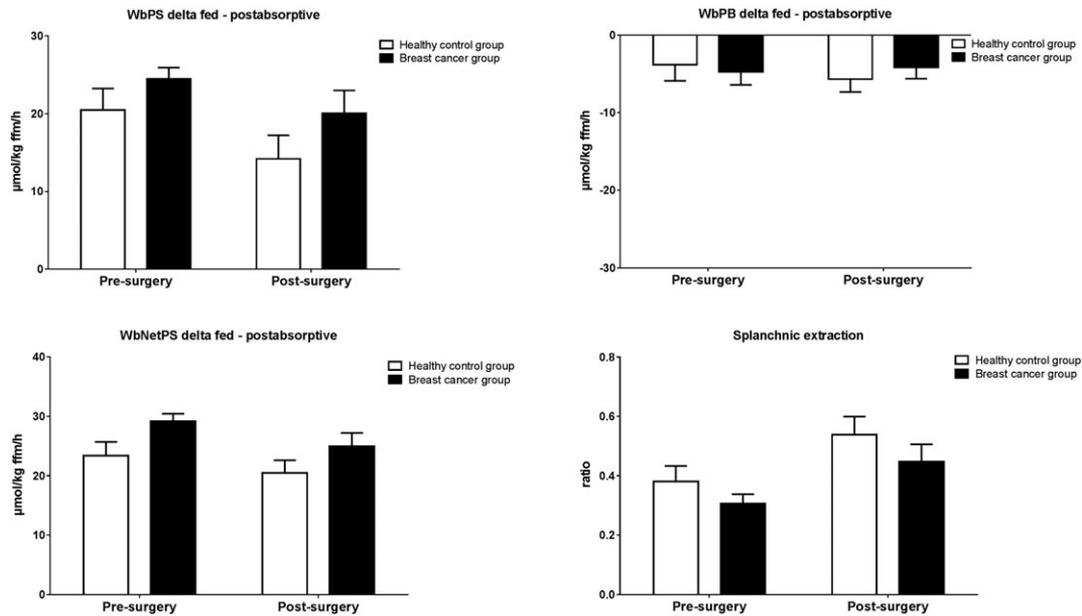
Figure 1 Mean (\pm SEM) kinetics of postprandial amino acid appearance in the systemic circulation from the meal (=exogenous rate of appearance corrected for extraction of amino acids from the meal by the splanchnic area) [pre-surgery (A) and post-surgery (B)], net protein anabolism [pre-surgery (C) and post-surgery (D)], and sum of all amino acid concentrations [pre-surgery (E) and post-surgery (F)] in the breast cancer (dotted line, closed circles) and the healthy control (straight line, open circles) groups. A separate pre surgery and post-surgery figure was made for each variable for illustrative purpose only. Two-factor repeated-measures ANOVA was used for each variable to test surgery (S) and group (G) effect. Postprandial amino acid appearance in the systemic circulation from the meal: surgery effect ($P < 0.001$), net protein anabolism: surgery ($P < 0.05$) and group effect ($P < 0.05$), sum of all plasma amino acid concentrations: surgery effect: $P < 0.001$. There was no surgery \times group interaction.



surgery.²⁸ An increased acute phase protein synthesis, related to the enhanced systemic inflammatory response, might therefore contribute to the observed increase in whole body protein synthesis and reduced net protein catabolism as obtained by the combined phenylalanine and tyrosine tracers. The observed reduced overnight net protein loss was confirmed independently by infusing the ¹³C-urea stable isotope showing lower values for whole body urea synthesis and plasma urea concentrations after surgery. Our subjects were studied within 24 h post-surgery, indicating that they were in the early catabolic phase. The

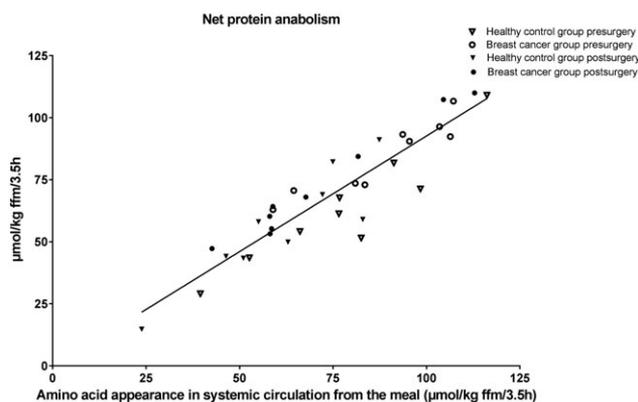
observed elevated whole body protein turnover and reduced net protein loss in the post-absorptive state are in line with those reported in the acute model of endotoxin-induced inflammation in healthy subjects.²⁹ This model elicits many of the systemic hormonal and substrate changes associated with surgical injury, including insulin resistance and increased stress response of hormones, known to increase protein breakdown and hepatic ureagenesis.³⁰ The increased protein synthesis in this model was explained by an increased splanchnic bed amino acid uptake in relation to increased acute phase protein synthesis.³¹ Although

Figure 2 Change (mean \pm SEM values) in protein synthesis (A), protein breakdown (B), net protein anabolism (C) after meal intake (difference between postprandial and post-absorptive values), and mean (\pm SEM) of splanchnic extraction of dietary amino acids (D) in the breast cancer group (black bar) and the healthy control group (open bar) before and within 24 h after surgery. Two-factor repeated-measures ANOVA was used to test surgery (S) and group (G) effect. Protein synthesis: surgery effect ($P < 0.05$) and a tendency towards a group effect ($P = 0.08$), net protein anabolism: surgery ($P < 0.01$) and group effect ($P < 0.05$), splanchnic extraction: surgery effect: $P < 0.001$. There was no surgery \times group interaction.



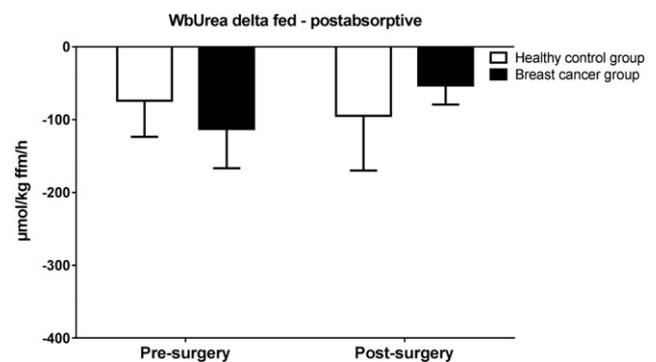
the exact location of the observed surgery induced up-regulation of whole body protein turnover remains unclear, it is likely that the increased protein breakdown is

Figure 3 Correlations between net protein anabolism (expressed in $\mu\text{mol/kg FFM}/3.5 \text{ h}$) and amino acid appearance in the systemic circulation from the meal (=exogenous rate of appearance corrected for extraction of amino acids from the meal by the splanchnic area) in the breast cancer group [pre-surgery (open circles), post-surgery (closed circles)] and healthy control group [pre-surgery (open triangles), post-surgery (closed triangles)], after intake of the high protein supplement. A highly significant relationship was found ($R^2 = 0.85$, $P < 0.001$) that remained after stratification for group (cancer vs. controls) and surgery (pre-surgery vs. post-surgery). Data were analysed with two-tailed tests of significance by using Pearson's correlation coefficients (breast cancer: $n = 9$, healthy controls: $n = 9$).



taking place in the splanchnic as well as in the muscle compartment. A previous study in unmedicated females undergoing elective total abdominal hysterectomy²⁵ showed that whole body protein breakdown did not change with general anaesthesia but decreased with both surgery and anaesthesia, and during the acute recovery period. It remains unclear whether the combination of multiple factors (including medication, starvation, anaesthesia, trauma

Figure 4 Change (mean \pm SEM values) in whole body urea synthesis after meal intake (difference between postprandial and post-absorptive value) in the breast cancer group (black bar) and the healthy group (open bar) pre-surgery and post-surgery. There was no group or surgery effect and no surgery \times group interaction.



severity) contributes to our observed up-regulated protein turnover after surgery.

Major surgery resulted in a reduced anabolic response to a meal due to a lower response in protein synthesis after surgery, whereas no difference was observed in protein breakdown. A reduced anabolic response to meal intake after surgery was most likely related to the increased splanchnic extraction of dietary amino acids, leading to a lower systemic amino acid availability for muscle protein synthesis. Post-surgery, the amino acid appearance in the systemic circulation reached a lower but longer lasting plateau, indicating that the anabolic effects of the meal are not completed 3.5 h after meal intake. Factors such as delayed gastric emptying and/or absorption/digestion problems, often present after surgery, might be responsible for this delayed uptake of food-derived amino acids. We hypothesize that the increased splanchnic extraction of dietary amino acids after surgery is needed for splanchnic protein synthesis (e.g. liver) that is particularly important when systemic inflammation is high and insulin sensitivity is reduced, further enhancing protein breakdown. This suggests that the quality of the proteins in the nutritional supplement and/or the total volume of intake were not high enough to sufficiently increase the plasma amino acid concentration to prevent the reduction in the anabolic response 24 h after surgery. Alternatively, it is very possible that the digestibility of the proteins in the nutritional supplement is compromised, suggesting that incorporation of hydrolysed (fast) proteins as part of postoperative nutrition would benefit the protein anabolic response after surgery. Although, on average, whole body urea synthesis was lower in response to feeding, the significant surgery effect we observed for net protein gain by using the phenylalanine and tyrosine tracer methodology was not found when using the urea tracer method. Although the exact reasons are unclear, it might be related to the lack of steady state in urea enrichment prior to meal intake post-surgery and the fact that the urea pool is much larger than the phenylalanine and tyrosine pools, making the detection of a small change on top on a large urea turnover more difficult. This suggests that the phenylalanine and tyrosine isotope method is much more sensitive than the urea isotope in detecting meal-induced changes after surgery.

Cancer vs. controls

The patients with non-cachectic early stage breast cancer showed a comparable reduction in overnight catabolic response after surgery as the control group. Moreover, the observed higher values for net protein anabolism up until 3.5 h after meal intake in the cancer group post-surgery is in line with the higher amino acid appearance in the systemic circulation and the higher plasma amino acid concentration. A

recent human study in colorectal cancer also observed an improved anabolic response to a commercial amino acid infusion in patients with cancer within 48 h of undergoing resection surgery, although no control group was studied.¹¹ Extraction of dietary amino acids by the splanchnic area was not lower in the cancer group suggesting that other (disease specific) factors are responsible for the increased anabolic response to meal intake that may lead to less protein loss after surgery.

No anabolic resistance or attenuated anabolic response to the meal was present in non-cachectic early stage breast cancer prior to or after surgery, as shown by the highly significant linear relationship between net protein anabolism and the amount of dietary amino acids available in the systemic circulation. Our previous studies in non-small cell lung cancer and malnourished cystic fibrosis^{5,32} support the concept that dietary amino acids are anabolic stimuli for the peripheral skeletal compartment, even in chronic disease states.

The greater net anabolic response in the cancer group was related to a higher response in protein synthesis to the meal as no difference was observed in protein breakdown. The increased acute phase protein synthesis is a part of whole body protein synthesis rate whether a subject is in the post-absorptive state or in the postprandial state. Fibrinogen synthesis was elevated in fasting weight-losing patients with pancreatic cancer with an acute phase response (CRP > 10 mg/L)³³ as compared with healthy controls, and stimulation of albumin and fibrinogen synthesis with feeding was observed in the cancer group. Stimulation of albumin but not of fibrinogen synthesis was found in the control group in the fed state.³⁴ To what extent the enhanced net anabolic response to feeding in our weight-stable breast cancer is related to an elevated stimulation of liver protein synthesis needs to be examined.

Furthermore, our study revealed a highly significant linear relationship between net protein anabolism after meal intake and the amount of dietary amino acids available in the systemic circulation. The higher amino acid appearance in the systemic circulation in cancer may be related to a lower splanchnic extraction of dietary amino acids (like previously observed in chronic obstructive pulmonary disease³⁵) although significance was not obtained in the present study likely due to the small study groups. Besides more insight in 24 h protein kinetics and net balance after surgery, more research is needed to unravel the contribution of the gut, liver, and muscle compartment to the enhanced protein anabolic response to feeding in weight-stable patients with breast cancer.

Limitations of the study are the small sample size although sufficient to answer our research aim as we used very precise methods to measure protein kinetics and that there was some heterogeneity among the subjects. As we carefully selected patients with non-cachectic weight-stable early stage breast cancer, we do not know whether patients with

advanced cancer undergoing the same surgical procedure would respond with similar (higher) anabolic response to meal intake. Furthermore, subjects were studied the day after surgery to minimize the risk of losing them due to early hospital discharge. By focusing on the acute (24 h) metabolic response to surgery, the maximal catabolic effect of surgery might not be reached in this time period due to ongoing systemic hormonal and substrate changes including inflammatory response, insulin resistance, and stress response of hormones. To accurately determine the impact of a major surgical procedure on the protein metabolic response, the patients should be studied over several time points during the recovery phase. Furthermore, as the anabolic effects of the meal are not completed 3.5 h after meal intake in both groups, it remains unclear whether the higher anabolic effect of feeding in breast cancer remains present when blood was sampled for a longer duration (>3.5 h) after food intake.

To assure that the oral phenylalanine stable isotope tracer that was added to the meal has the same absorption pattern than the amino acids from the digested protein that are absorbed, we plotted the pattern of the tracee (from the digested protein) and that of the tracer (the added stable tracer) after meal intake, both pre-surgery and post-surgery (Supporting Information, Figure S5). The patterns of the tracee and the tracer were the same indicating that adding a free amino acid isotope to a complete meal (in our case, a bottle of BOOST) enables measurement of net protein anabolism after meal intake.

In conclusion, the acute catabolic response to major surgery and reduced anabolic response to feeding is not enhanced in non-cachectic weight-stable patients with early stage breast cancer. In fact, the higher anabolic response to feeding in cancer was indicative of a more preserved meal efficiency, which may act as an early compensatory mechanism to reduce protein loss and preserve protein mass after major surgery. The anabolic potential to conventional feeding therefore remains exploitable in patients with non-cachectic early stage cancer within 24 h after major surgery.

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Online supplementary material

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Overview experimental study design for both study days for the breast cancer patients and healthy controls. Body composition, skeletal muscle function and respiratory muscle strength were only assessed on the first study day

Figure S2. Mean (\pm SEM) of plasma C-reactive protein (CRP) (Figure S2a), Glucose (Figure 2b), Insulin (Figure S2c) and HOMA index (Figure S2d) in the Breast cancer group (black bar) and the healthy control group (open bar) in the post-absorptive state before and after surgery. Two-factor repeated-measures ANOVA was used to test Surgery (S) and group (G) effect. CRP: Surgery effect: $p < 0.001$, glucose: Surgery effect ($p < 0.01$). HOMA: Surgery effect: $p < 0.01$. There was no cancer effect or Surgery X Group interaction.

Figure S3. Mean (\pm SEM) plasma enrichment kinetics expressed as tracer-tracee ratio corrected for background enrichment (cTTR) of phenylalanine (PHE)5, tyrosine (TYR)4, TYR2, and UREA1 before and after intake of the meal at $t = 90$ min in the breast cancer (dotted lines) and healthy control (straight lines) groups, both pre-surgery (closed circles) and post-surgery (open circles).

Figure S4. Mean (\pm SEM) kinetics of protein synthesis and breakdown before and after intake of the meal at $t = 90$ min in the breast cancer (dotted lines) and healthy control (straight lines) groups, both pre-surgery (closed circles) and post-surgery (open circles). A separate pre- and post-surgery figure was made for each variable for illustrative purpose only. Two-factor repeated-measures ANOVA was used for each variable to test Surgery (S) and group (G) effect. Protein synthesis: surgery effect ($P < 0.01$), protein breakdown: surgery ($P < 0.001$). There was no Group or Surgery X Group interaction.

Figure S5. Plasma phenylalanine concentration of the unlabeled tracee coming from the digested protein (circles) and from the oral tracer added to the meal (triangles), in the breast cancer (dotted lines) and healthy control (straight lines) groups both pre-surgery (left panel) and post-surgery (right panel).

Table S1. Infusion rates of stable isotopes.

Table S2. Characterization of the individual study participants

Conflict of interest

none declared.

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