Published in final edited form as:

Clin Nutr. 2018 October ; 37(5): 1645–1653. doi:10.1016/j.clnu.2017.07.019.

Major surgery diminishes systemic arginine availability and suppresses nitric oxide response to feeding in patients with early stage breast cancer

Mariëlle P.K.J. Engelen^{a,b}, V. Suzanne Klimberg^c, Arianna Allasia^b, and Nicolaas E.P. Deutz^{a,b}

^aCenter for Translational Research on Aging and Longevity, Dept. of Health and Kinesiology, Texas A&M University, College Station, TX, USA

^bDept. of Geriatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA

^cDivision of Breast Surgical Oncology, Dept. of Surgery, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Abstract

Background and aims—Plasma arginine (ARG) levels are reduced in breast cancer, suggesting diminished systemic ARG availability. ARG and its product nitric oxide (NO) are important in early postoperative recovery due to its roles in immune function and wound healing. It remains unclear whether major surgery further diminishes systemic ARG availability due to enhanced ARG catabolism and/or insufficient endogenous ARG synthesis negatively affecting NO synthesis in patients with early stage breast cancer.

Methods—In 9 women with early stage breast malignancy and 9 healthy women with genetic predisposition to breast cancer, whole body ARG and citrulline (CIT) rates of appearances were measured to determine their production rates prior to and within 24h after major breast surgery by stable isotope methodology in the postabsorptive and postprandial state. The conversions of CIT>ARG, ARG>CIT, and ARG>Urea (markers of *de novo* ARG and NO synthesis, arginase activity, respectively), and ARG clearance (reflecting ARG disposal capacity) were calculated.

Results—Prior to surgery, plasma ARG, CIT and glutamine concentrations were lower in cancer ($P<0.05$) but no differences were found in the rate of appearances of ARG, CIT and their

Corresponding author. Mariëlle PKJ Engelen, PhD, Center for Translational Research in Aging & Longevity, Dept. of Health and Kinesiology, Texas A&M University, Suite 210. 1700 Research Parkway, College Station, TX 77843-4253. mpkj.engelen@ctral.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author contributions

MPKJE and NEPD designed the research. MPKJ, AA and VSK recruited the subjects and conducted the research. MPKJE and NEPD performed data analysis, had full access to all of the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis. MPKJE, NEPD and VSK wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflicts of interest.

This trial was registered at clinicaltrials.gov as NCT00497380

conversions. Surgery increased ARG clearance and reduced CIT rate of appearance, conversion of CIT>ARG ($P<0.001$), and plasma ARG, CIT, ornithine concentrations ($P<0.001$). Furthermore, postprandial increase in ARG>CIT conversion ($p<0.05$), plasma ARG ($P<0.001$) and CIT ($P=0.06$) concentrations were lower after surgery. The cancer group had lower values for postprandial increase in ARG>CIT conversion, plasma CIT ($P<0.05$) and glutamine concentrations ($p=0.08$).

Conclusions—Major surgery in early stage breast cancer further reduces systemic ARG availability in the early phase of recovery due to a combined process of increased ARG catabolism and impaired endogenous ARG synthesis. The suppressed postprandial NO increase in early stage cancer suggests that specific nutritional approaches are advised to increase ARG availability after major surgery although the effects on postoperative recovery remain unclear.

Keywords

Breast surgery; Early stage breast cancer; Arginine kinetics; Nitric oxide synthesis; Stable isotopes

1. Introduction

A large proportion (91%) of breast cancer patients is characterized by a reduced plasma arginine level (ARG) (1, 2). ARG plays an important role in the immune function and in defense against tumor cells, and is used for whole body protein and urea synthesis (3). Reduced plasma ARG levels in cancer patients are irrespective of tumor stage and type, and nutritional status (1), suggesting that a diminished systemic ARG availability can be found in weight stable breast cancer patients early in their disease trajectory.

Circulating myeloid derived suppressor cells (MDSCs) are elevated in patients with breast cancer (4) (5), and even in early stage breast malignancy (6). MDSC are potent suppressors of tumor immunity, and are able to deplete ARG levels and alter nitric oxide (NO) production by producing the ARG catabolic enzymes arginase and inducible nitric oxide synthase (iNOS). Elevated arginase activities in serum and human breast tissue samples have been found in breast cancer (7, 8), suggesting that increased systemic and local (tumor) ARG catabolism play a role in the reduced ARG availability in these patients. Recently, we observed in advanced non-small cell lung cancer (NSCLC) patients that the reduced systemic ARG availability is related to a compromised endogenous ARG production from its precursor citrulline (CIT) (9) despite an elevated protein breakdown (10), and was associated with reduced whole body NO synthesis (9). It remains unclear whether the reduced plasma ARG level in breast cancer patients (1, 2) is the consequence of insufficient endogenous ARG production from CIT to balance the increased ARG catabolism.

ARG also plays an important role in postoperative recovery in cancer as it is required for adequate NO synthesis necessary for immune function, cell regeneration, tissue perfusion, and wound healing (11). Low plasma ARG levels are present in patients with infected wounds, and impaired wound healing is related to altered arginine usage (12). We previously observed that a tumor prohibits the normal postoperative increase in whole body ARG production (13) and reduces endogenous (*de novo*) ARG production from citrulline (14) after surgery, in mice indicating a compromised postoperative ARG metabolism. Major

surgery in early stage breast cancer resulted in a preserved upregulation of protein breakdown (15), suggesting that their ARG production from protein breakdown is not attenuated by surgery. We therefore hypothesize that systemic ARG deficiency in patients with early stage breast cancer is enhanced by major surgery and mainly due to reduced endogenous ARG synthesis, negatively affecting NO synthesis.

The aim of the present study was to investigate whether the reduced systemic arginine availability in early stage breast cancer is due to enhanced ARG catabolism and/or reduced endogenous ARG synthesis, and to examine the effects of major breast surgery. Combined bilateral mastectomy and reconstruction surgery was chosen as it is a common and comparable major surgical procedure in women with breast cancer and in healthy women with a genetic predisposition to breast cancer. Arginine, citrulline and NO kinetics were measured in both groups prior to and within 24h post-surgery using stable isotope techniques in the overnight fasted state and after intake of a conventional medical food supplement. Insight in the independent and combined effects of surgery and cancer on whole body arginine metabolism and NO synthesis may initiate novel nutritional approaches for cancer patients undergoing major surgery to enhance postoperative recovery.

2. Subjects and methods

2.1. Subjects

Women with Stage II breast cancer and healthy control women with a genetic predisposition to breast cancer scheduled for major breast surgery participated in the study (15). All women were recruited during visits at the Women's Oncology Clinic Winthrop Rockefeller Cancer Institute at the University Arkansas for Medical Sciences and studied between November 2010 and March 2012. Exclusion criteria were neoadjuvant therapy and any surgery less than 4 weeks before the study, pre-existent cardiovascular disease or untreated metabolic disease, including liver or renal disease. Written informed consent was obtained from all subjects and the study was approved by the Institutional Review Board, and the Protocol Review and Monitoring Committee of the Arkansas Cancer Research Center, University Arkansas for Medical Sciences, Little Rock, AR.

2.2. Breast Surgery Procedures

Patients with invasive breast cancer and healthy control subjects scheduled for bilateral mastectomy – with or without lymphadenectomy – which was immediately followed by reconstruction surgery were invited to participate (15). All patients underwent total skin sparing mastectomy. Initial reconstruction consisted of placement of pectoralis submuscular tissue expanders or implants. The bilateral mastectomy procedures were performed by the same breast cancer surgeon whereas the reconstruction surgeries were performed by two plastic surgeons using identical techniques. General anesthesia included opioid analgesics, and antibiotics, antiemetics, laxatives were provided to all patients and healthy subjects during surgery and/or post-surgery. Disease and treatment history, and surgery details were obtained from the subject's medical chart.

2.3. Study protocol

All breast cancer patients and healthy control 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. The metabolic part of the two study days was identical and lasted 5.5 hr (15). In the early morning after an overnight fast, body weight, height and body composition by Dual-energy X-ray Absorptiometry (Hologic QDR 4500/Version 12.7.3.1 (Bedford, MA)) were measured. The anthropometric and body composition data were standardized for height (16). Subsequently, for infusion of the stable isotopes, a peripheral line was placed in an antecubital vein of the arm. To measure ARG, CIT and NO kinetics, a primed, constant and continuous infusion was performed of the stable isotopes L-[guanidine- $^{15}\text{N}_2$]ARG (prime: $3.75\mu\text{mol/kg bw}$, infusion rate: $3.75\mu\text{mol/kg bw *h}$) and L-[ureido- ^{13}C - $^2\text{H}_2$] or L-[5- ^{13}C , $^2\text{H}_4$]CIT (prime: $0.88\mu\text{mol/kg bw}$, infusion rate: $0.30\mu\text{mol/kg bw *h}$), 1- ^{13}C -Urea (prime: $17.5\mu\text{mol/kg bw}$, infusion rate: $5.0\mu\text{mol/kg bw *h}$) (Cambridge Isotopic Laboratories (Woburn, MA, USA)). Before intravenous administration of the priming dose of stable isotopes, a venous blood sample was collected to measure background enrichments. A second catheter for arterialized venous blood sampling was placed in a superficial dorsal vein of the hand or lower arm of the contralateral arm. The hand was placed in a thermostatically controlled hot box, a technique to mimic direct arterial sampling (17). After 1.5 hours of intravenous infusion, each subject ingested within 5 minutes a commercial medical food (Boost-HP drink (8fl oz, 15g protein, 240 kcal, 30% protein/11% carbohydrates/9% fat)). Blood samples were taken throughout the study and until 3.5 h after intake of the supplement.

2.4. Biochemical analysis

Blood was processed, stored at -80°C , and analyzed batch-wise. Stable isotope enrichments and plasma amino acid concentrations were analyzed by LC-MS/MS (18).

2.5. Calculations for arginine and citrulline kinetics, and nitric oxide synthesis

Whole body ARG and CIT rates of appearances, CIT>ARG conversion (indirect measure of *de novo* ARG synthesis), ARG>CIT conversion (indirect indicator of NO synthesis), and ARG>Urea conversion (reflecting arginase activity) in plasma were calculated from isotope enrichment values as previously described (9, 19–21). Plasma ARG clearance was calculated as whole body ARG rate of appearance divided by plasma ARG concentration, and is the volume of plasma that is cleared of ARG per unit of time (reflecting the ARG disposal capacity of the body). Tracer-tracee ratio of ARG and CIT reached an isotopic steady state within the first 1.5 hours of infusion in both groups while in the postabsorptive state.

2.6. 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 and metabolic characteristics between the breast cancer and healthy control groups. Postabsorptive whole body ARG and CIT rate of appearance, CIT>ARG conversion, ARG>CIT conversion, and ARG>Urea were measured as the median value of the measures at the time points 70, 80 and 90 min, and in the postprandial state as the 3.5

hours integral after intake of the meal and expressed per hour ($\mu\text{mol/kg FFM}/3.5\text{h}$) and for the plasma amino acid concentrations as the mean postprandial concentrations (μM). Two-way repeated-measures analysis of variance (two way RM ANOVA; general linear model) was carried out with Group (breast cancer versus healthy controls) and Surgery (pre-surgery vs. post-surgery) as factors. In case of significant interactions between Surgery and Group, Bonferroni post hoc test was applied. The level of significance was set at $p < 0.05$. Graphpad Prism (Version 7.02) and SPSS (version 22) were used for data analysis.

3. Results

Thirteen breast cancer (Stage II) and eleven healthy control subjects were enrolled in the study. Three cancer subjects and one control subject did not meet the inclusion criteria, one cancer and one control subject dropped out because of time commitment of the first study day. Nine breast cancer and 9 healthy control subjects completed the study. No significant differences were found between the breast cancer and healthy control groups in age (50 ± 10 y vs. 49 ± 8 y, respectively), BMI (29 ± 5 kg/m^2 vs. 28 ± 6 kg/m^2 , respectively), fat-free mass index (16 ± 4 kg/m^2 vs. 17 ± 2 kg/m^2 , respectively) and fat mass index (12 ± 4 kg/m^2 vs. 12 ± 4 kg/m^2) (15). No unintentional weight loss was present in both groups, and none of the subjects were anorexic or were undergoing a restrictive diet.

3.1 Whole body arginine metabolism pre-surgery in the breast cancer and healthy control groups

In the postabsorptive state and pre-surgery (Table 1), lower concentrations were found for plasma ARG ($p < 0.01$), CIT and GLN ($p < 0.05$) in breast cancer patients as compared to the healthy controls but no difference was found in plasma ORN concentration. These changes could not be explained by alterations in whole body ARG kinetics as whole body rate of appearance of ARG and CIT, and the conversion of CIT>ARG (marker of *de novo* ARG synthesis), ARG>CIT (reflecting NO synthesis), ARG>Urea (marker of arginase activity), and ARG clearance (Table 1) were not different between the groups. Also in the postprandial state, lower concentrations were found in breast cancer for plasma ARG ($p < 0.01$), CIT ($p < 0.05$) and a tendency towards lower values for GLN ($p = 0.08$) whereas no differences were found for whole body ARG or CIT kinetics or conversions between the groups pre-surgery.

3.2 Clinical effects of breast surgical procedure in breast cancer patients and healthy controls

Although 2 out of 9 cancer patients did not choose to undergo reconstruction surgery, no significant differences were found in the duration of the surgical procedure (cancer: 430 ± 163 min. vs. controls: 442 ± 64 min.) or in the amount of blood loss (cancer: 343 ± 144 ml. vs controls: 210 ± 163 ml.). Surgery resulted in higher values for plasma CRP ($P < 0.001$) (breast cancer: presurgery: 1.1 ± 0.4 mg/L vs. post-surgery: 42.9 ± 8.5 mg/L; healthy controls: pre-surgery: 3.0 ± 1.8 mg/L vs. post-surgery: 34.4 ± 4.0 mg/L) and HOMA index (marker of insulin resistance) ($P < 0.01$) (breast cancer: presurgery: 1.9 ± 0.1 vs. post-surgery: 4.9 ± 1.0 ; controls: 1.4 ± 0.7 vs. 4.1 ± 1.6). No differences were observed in plasma hemoglobin level, white blood cell count lymphocyte, or platelet count before and after

surgery between the groups. The nutritional supplement was well tolerated as only one subject felt slightly nauseous after intake. All subjects were taking antiemetics as part of their postoperative care.

3.3 Effects surgery on postabsorptive arginine metabolism and nitric oxide synthesis

Surgery resulted in a reduction in whole body rate of appearance of CIT and CIT>ARG conversion ($P<0.001$), and in an increase in ARG clearance ($P<0.01$) (Table 1). Furthermore, surgery resulted in a reduction in plasma ARG, CIT, and ORN concentrations ($P<0.001$). There was an increase after surgery for ARG to ORN ratio ($P<0.01$). Lower levels were observed in the cancer for plasma ARG ($P<0.001$) and GLN ($P<0.05$) concentrations. Furthermore, there was a surgery X group interaction for CIT rate of appearance, ARG>CIT conversion, and plasma CIT concentration ($P<0.05$) related to the lower surgery induced changes in the breast cancer than in the control group ($P<0.05$). No significant relationships were observed between surgery induced change in CRP, and changes in arginine metabolism or nitric oxide synthesis.

3.4 Effects of surgery on the postprandial responses to feeding of whole body arginine metabolism and nitric oxide synthesis

Changes in time of postprandial kinetics of ARG, CIT and the conversions (Figure 1), and amino acid concentrations (Figure 2) are presented both pre- and post-surgery. The peak in ARG rate of appearance (Figure 1) was reached around 30 minutes after meal intake, and normalization toward baseline levels occurred 2 – 2.5 hours after meal intake. CIT rate of appearance, CIT>ARG and ARG>CIT conversions gradually increased after meal intake and reached a plateau around 2 hours after meal intake. Reduction towards normal values didn't take place even at 3.5h after meal intake (last blood sample of the study). The peak in plasma ARG and GLN concentrations (Figure 2) occurred around 45 min after meal intake. In line with the response observed for CIT rate of appearance, plasma CIT concentration prior to surgery gradually increased after meal intake and reached a plateau around 2 hours after meal intake. The plateau in plasma ORN was obtained at 1 hour after meal intake. Reduction towards normal values didn't take place for plasma CIT and ORN before the end of the study. The meal-induced changes in arginine metabolism (difference between postprandial and postabsorptive values) are presented in Figures 3 and 4. Surgery resulted in lower values for meal induced increase in ARG>CIT conversion ($p<0.05$) and CIT rate of appearance ($p=0.06$), as well as in plasma ARG ($p<0.001$), ORN ($p<0.01$) and CIT ($p=0.06$). Furthermore, the cancer group had lower values for meal induced increase in ARG>CIT conversion, and plasma concentrations of CIT ($p<0.05$) and GLN ($p=0.08$). No group $\times$ surgery effects were observed.

4. Discussion

In the present study, reduced plasma ARG levels were found in patients with early stage breast cancer indicative of diminished systemic ARG availability. Major surgery resulted in a further reduction in systemic ARG availability in the early phase of recovery, which was related to a combined process of increased ARG catabolism and impaired endogenous ARG synthesis, without affecting nitric oxide synthesis in the postabsorptive state. The suppressed

meal induced NO increase particularly in the breast cancer group suggests that specific nutritional approaches could be of benefit to restore systemic ARG availability after major surgery.

4.1 Reduced postabsorptive plasma ARG concentration in breast cancer prior to surgery

The observed reduction in plasma ARG, CIT and GLN concentrations in our weight-stable cancer group are in line with previous findings that alterations in plasma amino acid profile are present in early stage cancer without evidence of cachexia, recent involuntary weight loss or enhanced systemic inflammation (22). Furthermore, the lower plasma ARG levels in breast cancer patients are in line with previous data in (mainly stage II) breast cancer (1, 23). The increased MDSCs in breast cancer (4, 5), even in early stage malignancy (6), may contribute to depletion of plasma ARG levels through enhanced ARG catabolism. Previously enhanced arginase activity levels were observed in human breast tissue (24) and serum of patients with breast cancer (7, 8), and the levels of serum arginase activity were positively correlated with advanced stages of breast cancer (8). In our early stage breast cancer patients, ARG clearance and plasma arginase activity assessed by stable isotope methodology were not elevated. A difference in disease severity, sampling location (tissue, serum/plasma) and/or method to assess arginase activity (biochemical vs. stable isotope methodology) may play a role in this. Recently, we found a comparable whole body protein breakdown rate in early stage breast cancer and healthy control women (15), suggesting no changes in ARG appearance coming from protein breakdown. The fact that besides plasma ARG also GLN and CIT concentrations are reduced in early stage breast cancer patients suggests an ARG precursor problem in this group of patients.

4.2 Metabolic effects of surgery

So far, no human studies are available unraveling the (individual and combined) effects of a major surgical procedure and the presence of early stage breast cancer on whole body ARG and CIT kinetics, and NO synthesis. The performed breast surgery was a major surgical procedure inducing severe metabolic stress within 24 hours post-surgery, as reflected by the enhanced systemic inflammation and insulin resistance. However the systemic inflammation post-surgery was still considerable lower than previously observed after other major surgery procedures (average CRP after major breast surgery versus gastrointestinal surgery(25): 40 vs 160 mg/L, respectively). Still the breast surgery resulted in a sharp fall of nearly all measured plasma amino acid concentrations including ARG (15), which is in line with the very low plasma ARG levels previously found on the first day after thoracoabdominal surgery (26).

The observed elevated ARG clearance post breast surgery, which reflects increased ARG catabolism, was not related to the elevated plasma arginase activity. In contrast, an increased arginase activity was found within a few hours after surgery in a murine model (27), and a tight negative correlation was observed between plasma ARG concentration and arginase activity. In the present study, whole body NO synthesis rate was not significantly increased post-surgery, indicating no stimulated conversion of ARG to CIT through the NOS pathway. In line, a previous study showed that iNOS activity in splenic immune cells was not elevated 24h after surgery or trauma but that only arginase I was increased (28). Moreover, our data

are in line with previous findings in node-positive breast cancer patients who had undergone surgery (29). In that study, an elevation was found in asymmetric dimethylarginine (ADMA), an endothelin and nitric oxide synthase inhibitor which is known to suppress NO synthesis.

Besides enhanced ARG catabolism, major surgery resulted in a reduced CIT appearance, ARG *de novo* synthesis, and plasma concentrations of ARG, CIT and ORN within 24h post-surgery, independent of the presence of breast cancer. In line, a rodent model showed reduced levels for plasma ARG and CIT within a few hours after surgery (27). Under normal conditions, the body is capable of producing sufficient ARG to satisfy metabolic requirements. However, pathophysiological circumstances such as enhanced systemic inflammation may arise in which endogenous ARG production is inadequate. The present study suggests that major surgery may be viewed as a metabolic stressor in which endogenous ARG production is inadequate, particularly in those with cancer.

4.3 Postprandial arginine metabolism

Major surgery resulted in a suppressed increase in plasma ARG, CIT, and ORN after intake of a conventional medical food supplement. This is in line with our recently reported suppressed anabolic response to the supplement after surgery (15), due to a reduced response in protein synthesis and suppressed increase in splanchnic extraction of dietary amino acids, leading to a diminished systemic amino acid availability. A lower meal induced increase in whole body NO synthesis was observed after surgery and particularly in those subjects with breast cancer. Previously in advanced NSCLC (9), we found that intake of a balanced mixture of amino acids was actually able to increase ARG rate of appearance and plasma ARG concentration, and restore NO synthesis.

The present data might suggest that the amino acid composition of the provided conventional medical food and/or the digestibility of the proteins in the supplement are not optimal for the early phase after surgery. Incorporation of hydrolyzed (fast) proteins or free amino acid mixture with high L-ARG or CIT levels as part of postoperative nutrition would likely benefit ARG metabolism and NO synthesis post-surgery. This may benefit particularly the cancer group as lower values were observed for the postprandial response in plasma CIT and GLN levels in these patients than in the control subjects. Furthermore, L-ARG supplementation has been reported to improve the immune system (30), thereby countering the suppressive effects of MDSC, and inhibit the progression of colon cancer (31). Dietary supplementation with ARG in breast cancer patients has been shown to enhance host defenses and therefore may have a role in adjuvant treatment (32). Furthermore, we previously observed that oral GLN intake is able to increase plasma GLN concentration in breast cancer patients and reduce radiation morbidity (33). Furthermore, oral GLN intake suppresses mammary carcinogenesis by activation of apoptosis in tumor cells (34). In arginine deficient head and neck cancer patients, perioperative treatment with ARG-rich supplements was able to improve long-term survival (35) and short-term outcomes, such as local wound complications and length of hospital stays (36). These studies suggest that restoring the reduced systemic ARG availability might be of clinical importance even in early stage non-cachectic cancer patients before undergoing major surgery.

4.4 Limitations of the study

The sample size was small but sufficient to answer our research aim as we used very precise methods to measure arginine and citrulline kinetics. The results that now appeared not significant with the small study groups might have become significant when more subjects had been included. As we studied early stage weight stable breast cancer patients, it remains unclear whether metastatic cachectic breast cancer patients would respond with a comparable metabolic response to surgery. Furthermore, the subjects were studied the day after surgery to minimize the risk of losing them due to early discharge. As very low plasma ARG levels have been observed in non-cancer patients lasting at least 6 days post-surgery (26), the time course of the alterations in ARG and NO kinetics post-surgery needs further investigation particularly in those with cancer.

5.0 Conclusions

Major breast surgery further reduces the systemic ARG availability in early stage breast cancer without affecting nitric oxide synthesis in the early phase of recovery due to a combined process of increased ARG catabolism and impaired endogenous ARG synthesis. The suppressed meal induced NO increase in the early stage non-cachectic breast cancer group after surgery suggests that specific nutritional approaches are advised to restore systemic ARG availability and NO kinetics. Whether this will positively influence their postoperative recovery needs further investigation.

Acknowledgments

We sincerely thank the breast cancer patients and the healthy control subjects who have participated in this research and made this work possible.

Funding

The project was supported by grant UL1RR029884 (Clinical and Translational Science Award) through the National Institutes of Health (NIH).

List of abbreviations

ARG	arginine
BMI	body mass index
CIT	citrulline
COPD	Chronic Obstructive Pulmonary Disease
CRP	c-reactive protein
FFMI	fat-free mass Index
GLN	glutamine
HOMA	Homeostasis Model Assessment
iNOS	inducible nitric oxide synthase

MDSC	myeloid derived suppressor cells
NO	nitric oxide
ORN	ornithine

References

1. Vissers YL, Dejong CH, Luiking YC, Fearon KC, von Meyenfeldt MF, Deutz NE. Plasma arginine concentrations are reduced in cancer patients: evidence for arginine deficiency? *Am J Clin Nutr.* 2005; 81(5):1142–6. [PubMed: 15883440]
2. Naini AB, Dickerson JW, Brown MM. Preoperative and postoperative levels of plasma protein and amino acid in esophageal and lung cancer patients. *Cancer.* 1988; 62(2):355–60. [PubMed: 3383136]
3. Morris SM Jr. Arginine: beyond protein. *Am J Clin Nutr.* 2006; 83(2):508S–12S. [PubMed: 16470022]
4. Ohki S, Shibata M, Gonda K, Machida T, Shimura T, Nakamura I, et al. Circulating myeloid-derived suppressor cells are increased and correlate to immune suppression, inflammation and hypoproteinemia in patients with cancer. *Oncology reports.* 2012; 28(2):453–8. [PubMed: 22614133]
5. Gonda K, Shibata M, Ohtake T, Yasuda M, Abe N, Watanabe K, et al. Myeloid-derived suppressor cells in patients with breast cancer. *Gan To Kagaku Ryoho.* 2012; 39(9):1363–8. [PubMed: 22996770]
6. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol Immunother.* 2009; 58(1):49–59. [PubMed: 18446337]
7. Porembska Z, Lubinski G, Chrzanowska A, Mielczarek M, Magnuska J, Baranczyk-Kuzma A. Arginase in patients with breast cancer. *Clinica chimica acta; international journal of clinical chemistry.* 2003; 328(1–2):105–11. [PubMed: 12559605]
8. Polat MF, Taysi S, Polat S, Boyuk A, Bakan E. Elevated serum arginase activity levels in patients with breast cancer. *Surg Today.* 2003; 33(9):655–61. [PubMed: 12928840]
9. Engelen MP, Safar AM, Bartter T, Koeman F, Deutz NE. Reduced arginine availability and nitric oxide synthesis in cancer is related to impaired endogenous arginine synthesis. *Clin Sci (Lond).* 2016; 130(14):1185–95. [PubMed: 27129191]
10. Engelen MP, Safar AM, Bartter T, Koeman F, Deutz NE. High anabolic potential of essential amino acid mixtures in advanced nonsmall cell lung cancer. *Ann Oncol.* 2015; 26(9):1960–6. [PubMed: 26113648]
11. Luiking YC, Ten Have GA, Wolfe RR, Deutz NE. Arginine de novo and nitric oxide production in disease states. *American journal of physiology.* 2012; 303(10):E1177–89. [PubMed: 23011059]
12. Debats IB, Booi D, Deutz NE, Buurman WA, Boeckx WD, van der Hulst RR. Infected chronic wounds show different local and systemic arginine conversion compared with acute wounds. *The Journal of surgical research.* 2006; 134(2):205–14. [PubMed: 16631201]
13. Vissers YL, von Meyenfeldt MF, Luiking YC, Dejong CH, Buurman WA, Deutz NE. Presence of tumour inhibits the normal post-operative response in arginine and NO production in non-cachectic mice. *Clin Sci (Lond).* 2007; 112(10):527–32. [PubMed: 17212586]
14. Vissers YL, von Meyenfeldt MF, Luiking YC, Dejong CH, Deutz NE. Interorgan synthesis of arginine is down-regulated in tumor-bearing mice undergoing surgical trauma. *Metabolism: clinical and experimental.* 2008; 57(7):896–902. [PubMed: 18555829]
15. Engelen MPKJ, Klimberg VS, Allasia A, Deutz NEP. Presence of early stage cancer does not impair the early protein metabolic response to major surgery. *Journal of cachexia, sarcopenia and muscle.* 2016 In press.

16. VanItallie TB, Yang MU, Heymsfield SB, Funk RC, Boileau RA. Height-normalized indices of the body's fat-free mass and fat mass: potentially useful indicators of nutritional status. *Am J Clin Nutr*. 1990; 52(6):953–9. [PubMed: 2239792]
17. Abumrad NN, Rabin D, Diamond MP, Lacy WW. Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism: clinical and experimental*. 1981; 30(9):936–40. [PubMed: 7022111]
18. Engelen MP, Com G, Wolfe RR, Deutz NE. Dietary essential amino acids are highly anabolic in pediatric patients with cystic fibrosis. *Journal of cystic fibrosis: official journal of the European Cystic Fibrosis Society*. 2013; 12(5):445–53. [PubMed: 23357545]
19. Engelen MP, Com G, Luiking YC, Deutz NE. Stimulated nitric oxide production and arginine deficiency in children with cystic fibrosis with nutritional failure. *The Journal of pediatrics*. 2013; 163(2):369–75. [PubMed: 23419590]
20. Luiking YC, Poeze M, Ramsay G, Deutz NE. Reduced citrulline production in sepsis is related to diminished de novo arginine and nitric oxide production. *Am J Clin Nutr*. 2009; 89(1):142–52. [PubMed: 19056593]
21. de Betue CTI, Garcia Casal XC, van Waardenburg DA, Schexnayder SM, Joosten KFM, Deutz NEP, et al. 24-Hour protein, arginine and citrulline metabolism in fed critically ill children – A stable isotope tracer study. *Clinical nutrition (Edinburgh, Scotland)*. 2017; 36(3):876–87.
22. Lai HS, Lee JC, Lee PH, Wang ST, Chen WJ. Plasma free amino acid profile in cancer patients. *Seminars in cancer biology*. 2005; 15(4):267–76. [PubMed: 15894488]
23. Alves CRR, da Cunha TF, da Paixao NA, Brum PC. Aerobic exercise training as therapy for cardiac and cancer cachexia. *Life Sciences*. 2015; 125:9–14. [PubMed: 25500304]
24. Singh R, Avliyakov NK, Braga M, Haykinson MJ, Martinez L, Singh V, et al. Proteomic identification of mitochondrial targets of arginase in human breast cancer. *PloS one*. 2013; 8(11):e79242. [PubMed: 24223914]
25. Lopez Hellin J, Baena-Fustegueras JA, Sabin-Urkia P, Schwartz-Riera S, Garcia-Arumi E. Nutritional modulation of protein metabolism after gastrointestinal surgery. *European journal of clinical nutrition*. 2008; 62(2):254–62. [PubMed: 17375114]
26. Nijveldt RJ, Prins HA, Siroen MP, Rauwerda JA, Teerlink T, van Leeuwen PA. Low arginine plasma levels in patients after thoracoabdominal aortic surgery. *European journal of clinical nutrition*. 2000; 54(8):615–7. [PubMed: 10951509]
27. Pribis JP, Zhu X, Vodovotz Y, Ochoa JB. Systemic arginine depletion after a murine model of surgery or trauma. *JPEN Journal of parenteral and enteral nutrition*. 2012; 36(1):53–9. [PubMed: 22179519]
28. Bernard AC, Mistry SK, Morris SM Jr, O'Brien WE, Tsuei BJ, Maley ME, et al. Alterations in arginine metabolic enzymes in trauma. *Shock*. 2001; 15(3):215–9. [PubMed: 11236905]
29. Alacacioglu A, Kebapcilar L, Sari I, Gokgoz Z, Tarhan O, Somali I, et al. Taxane-based adjuvant chemotherapy reduces endothelin-1 and symmetric dimethylarginine levels in patients with breast cancer. *J BUON*. 2010; 15(3):572–6. [PubMed: 20941830]
30. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol*. 2005; 5(8):641–54. [PubMed: 16056256]
31. Yeh CL, Pai MH, Li CC, Tsai YL, Yeh SL. Effect of arginine on angiogenesis induced by human colon cancer: in vitro and in vivo studies. *The Journal of nutritional biochemistry*. 2010; 21(6): 538–43. [PubMed: 19447018]
32. Cao Y, Wang Q, Du Y, Liu F, Zhang Y, Feng Y, et al. l-arginine and docetaxel synergistically enhance anti-tumor immunity by modifying the immune status of tumor-bearing mice. *Int Immunopharmacol*. 2016; 35:7–14. [PubMed: 27003114]
33. Rubio I, Suva LJ, Todorova V, Bhattacharyya S, Kaufmann Y, Maners A, et al. Oral glutamine reduces radiation morbidity in breast conservation surgery. *JPEN Journal of parenteral and enteral nutrition*. 2013; 37(5):623–30. [PubMed: 23389298]
34. Todorova VK, Harms SA, Kaufmann Y, Luo S, Luo KQ, Babb K, et al. Effect of dietary glutamine on tumor glutathione levels and apoptosis-related proteins in DMBA-induced breast cancer of rats. *Breast Cancer Res Treat*. 2004; 88(3):247–56. [PubMed: 15609127]

35. Buijs N, van Bokhorst-de van der Schueren MA, Langius JA, Leemans CR, Kuik DJ, Vermeulen MA, et al. Perioperative arginine-supplemented nutrition in malnourished patients with head and neck cancer improves long-term survival. *Am J Clin Nutr.* 2010; 92(5):1151–6. [PubMed: 20881073]
36. De Luis DA, Izaola O, Cuellar L, Terroba MC, Martin T, Aller R. High dose of arginine enhanced enteral nutrition in postsurgical head and neck cancer patients. A randomized clinical trial. *Eur Rev Med Pharmacol Sci.* 2009; 13(4):279–83. [PubMed: 19694342]

Figure 1.

Kinetics of whole body arginine rate of appearance (Fig 1a), citrulline rate of appearance (Fig 1b), citrulline to arginine conversion (indirect marker of arginine *de novo* synthesis (Fig 1c), arginine clearance (1d) and arginine to citrulline conversion (indirect marker of nitric oxide synthesis (1e) in the breast cancer group (grey lines) and healthy control group (black lines), both pre-surgery (straight lines) and post-surgery (dotted lines), after intake of the conventional nutritional supplement at t=90 min. Values are mean ($\pm$ S.E.M.).

Two-factor repeated-measures ANOVA was used to test the Group (G) and Surgery (S) effect. There was a Surgery effect for whole body arginine and citrulline rate of appearance, citrulline to arginine conversion ($P<0.01$). There was no Group effect or Surgery X Group interaction.

Figure 2.

Kinetics of plasma concentrations of arginine (Fig 2a), citrulline (Fig 2b), glutamine (Fig 2c), and ornithine (2d) in the breast cancer group (grey lines) and healthy control group (black lines), both pre-surgery (straight lines) and post-surgery (dotted lines), after intake of the conventional nutritional supplement at $t=90$ min. Values are mean ($\pm$ S.E.M.).

Two-factor repeated-measures ANOVA was used to test the Group (G) and Surgery (S) effect. There was a Surgery effect for plasma concentration of arginine, citrulline, glutamine, and ornithine ($P<0.001$). There was a Group effect for plasma concentration of arginine and glutamine ($P<0.05$). There was no Surgery X Group interaction.

Figure 3.

Change (mean $\pm$ SEM values) in whole body arginine rate of appearance (Fig 3a), citrulline rate of appearance (fig 3b), citrulline to arginine conversion (indirect marker of arginine *de novo* synthesis (Fig 3c), arginine clearance (Fig 3d), and arginine to citrulline conversion (indirect marker of nitric oxide synthesis (Fig 3e) after meal intake (difference between postprandial and postabsorptive values), in the breast cancer group (black bar) and the healthy control group (open bar) before and within 24 hours after surgery.

Two-factor repeated-measures ANOVA was used to test the Group (G) and Surgery (S) effect. There was a Surgery effect for arginine to citrulline conversion ($P<0.05$) and citrulline rate of appearance ($P=0.06$). There was a Group effect for arginine to citrulline conversion ($P<0.05$). There was no Surgery X Group interaction.

Figure 4.

Change (mean $\pm$ SEM values) in plasma concentration of arginine (Fig 4a), citrulline (fig 4b), glutamine (Fig 4c), and ornithine (Fig 4d) after meal intake (difference between postprandial and postabsorptive values), in the breast cancer group (black bar) and the healthy control group (open bar) before and within 24 hours after surgery.

Two-factor repeated-measures ANOVA was used to test the Group (G) and Surgery (S) effect. There was a Surgery effect for plasma concentration of arginine ($P < 0.001$), citrulline ($P = 0.06$) and ornithine ($P < 0.01$). There was a Group effect for plasma concentration of citrulline ($P < 0.05$) and glutamine ($P = 0.08$). There was no Surgery X Group interaction.

Table 1

Postabsorptive arginine metabolism and plasma amino acid concentrations prior to and within 24 hours after large breast surgery in the control and breast cancer groups¹

	Control group (n=9)		Breast cancer group (n=9)	
	Pre-surgery	Post-surgery	Pre-surgery	Post-surgery
Plasma amino acid concentrations				
ARG ^{5,7}	82 ± 3 ³	52 ± 4	61 ± 4	46 ± 3
CIT ^{5,9}	33 ± 1 ⁴	20 ± 1	28 ± 2	22 ± 2
GLN ⁸	711 ± 22 ⁴	691 ± 42	626 ± 27	580 ± 26
ORN ⁵	45 ± 2	25 ± 3	42 ± 4	29 ± 5
ARG/ORN ⁶	1.9 ± 0.1	2.0 ± 0.1	1.5 ± 0.1	1.7 ± 0.1
Amino acid kinetics				
ARG rate of appearance	71 ± 4 ²	69 ± 5	70 ± 2	68 ± 4
CIT rate of appearance ^{5,9}	11 ± 1	8 ± 1	10 ± 1	9 ± 1
CIT to ARG conversion ⁵	5.9 ± 0.7	4.2 ± 0.4	6.2 ± 0.7	5.0 ± 0.4
ARG to CIT conversion ⁹	0.10 ± 0.07	0.18 ± 0.23	0.13 ± 0.09	0.20 ± 0.11
ARG to Urea conversion	8.3 ± 0.5	8.6 ± 0.4	8.5 ± 0.4	9.2 ± 0.5
ARG clearance ⁶	16.9 ± 2.1	20.3 ± 2.2	15.9 ± 1.8	18.2 ± 2.0

¹ ARG: arginine, CIT: citrulline, GLN: glutamine, ORN: ornithine. CIT to ARG conversion is indirect marker of ARG *de novo* synthesis, ARG to CIT conversion is indirect marker of nitric oxide synthesis. ARG to Urea conversion is an indicator of arginase activity. Whole body postabsorptive ARG metabolism and plasma amino acid concentrations are expressed as the median value of the measures at time points 70, 80 and 90 min (in $\mu\text{mol/kg FFM/h}$ for the metabolic parameters, and μM for the plasma amino acid concentrations).

² Values are means ± S.E.M.

Unpaired Student's t test was used to determine pre-surgery differences between the breast cancer and healthy control groups. Two-factor repeated-measures ANOVA was used to test Surgery (S), Group (G) effect or Surgery × Group Interaction (I) *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

³ Statistical difference baseline (pre-surgery) between breast cancer and control groups: $P < 0.01$ by unpaired T-test

⁴ Statistical difference baseline (pre-surgery) between breast cancer and control groups: $P < 0.05$ by unpaired T-test

⁵ Surgery effect: $P < 0.001$

⁶ Surgery effect: $P < 0.01$

⁷ Group effect: $P < 0.001$

⁸ Group effect: $P < 0.05$

⁹ Surgery × Group interaction: $P < 0.05$