Arginine Improves Microcirculation in the Free Transverse Rectus Abdominis Myocutaneous Flap after Breast Reconstruction: A Randomized, Double-Blind Clinical Trial

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Background: Partial flap loss is caused by the incapability of the vascular pedicle to provide sufficient microvascular perfusion in distal segments of the flap in addition to the reperfusion injury that occurs in the whole flap after free tissue transfer. In experimental studies, the amino acid arginine reduces reperfusion injury and improves microvascular perfusion. The purpose of this clinical study was to explore the effect of arginine in free flap surgery.
Methods: In this randomized, double blind, placebo-controlled trial, 20 patients with unilateral breast reconstruction using the free transverse rectus abdominis myocutaneous flap were included. Patient and flap data were recorded. Patients received a continuous intravenous infusion of arginine or the control amino acid alanine for 5 days. Microcirculation was recorded in the flap in a standardized fashion using laser Doppler flowmetry (Perimed).
Results: Zone IV microcirculatory blood flow postoperatively was higher in the arginine group than in the alanine control group ($p = 0.04$).
Conclusion: The authors' study shows beneficial effects of intravenous therapy with arginine to improve microcirculation in the free transverse rectus abdominis myocutaneous flap. (*Plast. Reconstr. Surg.* 127: 2216, 2011.)

In recent decades, tremendous progress in free flap surgery has taken place.[1] Specifically, improved knowledge of vascular territories and flap design has significantly reduced free flap surgery–associated morbidity.[2] Despite these improvements, there is still considerable morbidity associated with either partial or total flap loss. Partial flap loss occurs in free flaps and pedicle flaps and is most commonly the result of microvascular perfusion failure.[3,4]

Partial flap loss is caused by the incapability of the vascular pedicle to provide sufficient microvascular perfusion in distal segments of the flap on top of the ischemia-reperfusion injury that occurs in the whole flap. The subsequent reperfusion injury results from increased leukocyte adhesion and migration into the capillaries, which leads to further tissue injury, increased microvascular permeability, and edema. In addition, elevated tissue pressure and inflammation will reduce microvascular perfusion and result in further deterioration and tissue loss. Because the amount of collateral microcirculation is less at greater distances from the vascular pedicle, the distal part of a free flap is more vulnerable to reduced microvascular circulation and ischemia-reperfusion injury. Therefore, specifically the distal parts of a free flap are affected by these processes and may lead to distal partial flap loss.[5]

Because of the superficial localization and accessibility for measurements, free flap surgery provides an ideal opportunity for clinical studies regarding reperfusion injury flap surgery. Several studies have been published with experimental models and have shown improved flap survival with either a systemic approach with pharmacologic interventions or with local procedures.[6–8]

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It is widely accepted that the arginine–nitric oxide pathway plays a pivotal role in microvascular perfusion and the pathophysiology of ischemia-reperfusion injury. Therefore, the nitric oxide pathway was studied in numerous studies in relation to its use in reducing ischemia-reperfusion injury.[9–14] The amino acid arginine is the sole precursor of nitric oxide. Arginine can be converted into nitric oxide and the amino acid citrulline by a family of tree isoforms of nitric oxide synthase.[15,16] Nitric oxide scavenges free radicals, released during reperfusion. Nitric oxide also is an important and potent vasodilator and prevents aggregation and activation of neutrophils and platelets.[17] Therefore, increasing nitric oxide production by stimulating the arginine–nitric oxide pathway may reduce the severity of ischemia-reperfusion injury. Earlier experimental studies in animals in which arginine was given intravenously showed a substantial reduction of ischemia-reperfusion injury in cutaneous and musculocutaneous flaps.[18,19]

Despite promising results in animals, data in humans are lacking. The free transverse rectus abdominis myocutaneous (TRAM) flap is used as a clinical surgical model with which to study the effect of arginine on microcirculatory blood flow and tissue viability. It is a frequently used free flap in breast reconstruction with well-established surgical technique. In addition, the patient population that undergoes this procedure is relatively homogenous, and the flap characteristics are constant.

PATIENTS AND METHODS

A randomized, double-blind, placebo-controlled clinical trial was performed that included 20 patients. The Ethics Committee of Maastricht University Medical Center approved the study protocol, and written informed consent was obtained from each subject. Patients were assigned randomly to arginine ($n = 10$) or placebo treatment ($n = 10$), by an independent clinical pharmacist, using numbered envelopes.

Patients included had a free TRAM flap for secondary breast reconstruction after mastectomy resulting from breast cancer. With this procedure, the lower abdominal fat and skin based on the deep inferior epigastric artery is transplanted and an anastomosis is made to the internal mammary artery to reconstruct the breast. The muscle-sparing procedure was chosen to reduce donor-site morbidity. After complete dissection, the flap is divided into four perfusion zones as reported previously.[20] Zone I is the central zone in the flap with the best perfusion, whereas zone IV is the most distal part with less collateral circulation and is usually the area at risk for insufficient perfusion and subsequent flap loss. This free flap was chosen because it is a frequently performed and standardized procedure, which ensured homogeneity between the participants. Patients were not admitted to the study if there was a history of previous midline laparotomy, which alters the lower abdominal circulation. Because the use of the total flap for reconstruction was considered relatively safe, zone IV was largely included in the reconstructed breast during the study.[21]

Patient medical history and demographics were recorded (Table 1). In addition, flap data such as total flap weight and weight used for breast reconstruction, percentage skin necrosis, and a disturbed wound healing in the outpatient clinic were recorded meticulously.

Based on literature at the initiation of the study, the most commonly used infusion dose of arginine was administered. Patients received intravenous supplementation of arginine or placebo (Bufa, Uitgeest, The Netherlands) prepared by

Table 1. Patient and Flap Characteristics

	All Patients (%)	Arginine (%)	Alanine (%)	*p*
Patient demographics (mean ± SEM)				
No. of patients	18	8	10	
Age, yr	48 ± 1	47 ± 3	43 ± 3	0.18*
Body weight, kg	71 ± 2	67 ± 4	71 ± 3	0.69*
BMI, kg/m^2	24 ± 1	27 ± 1	24 ± 1	0.13*
Length of stay, days	7 ± 0	7 ± 0	7 ± 0	0.71*
Flap records (mean ± SEM)				
Ischemia period, min	51 ± 4	54 ± 11	44 ± 3	1.00*
Total flap weight, g	962 ± 55	809 ± 111	999 ± 133	0.48*
Reconstructed breast weight, g	751 ± 47	669 ± 85	864 ± 79	0.12*
Risk factors				
Smoking	5 (28)	2 (25)	3 (30)	0.59†
Radiation	8 (44)	2 (25)	6 (60)	0.15†
Chemotherapy	13 (72)	4 (50)	9 (90)	0.09†

*Wilcoxon-Mann-Whitney *U* test (two-tailed).
†Fisher's exact test (two-tailed).

the local pharmacist. Intravenous supplementation consisted of either a daily dose of 30 g of arginine, dissolved in 1000 ml of 0.9% sodium chloride and adjusted to pH 7.2 using 10% hydrochloric acid (net nitrogen intake, 45.7 mM), or a placebo treatment consisting of a daily dose of 25.2 g of alanine, dissolved in 1000 ml of 0.9% sodium chloride (net nitrogen intake, 44 mM). The amino acid alanine was chosen to control for nitrogen intake and because alanine does not participate in the arginine–nitric oxide pathway. The infusions were made isovolumetric and isonitrogenous. Although it was not possible to make the infusions isocaloric, the daily arginine infusion accounted for 120 kcal and the placebo infusion accounted for 100.8 kcal. Patients received an infusion of 1000 ml in two doses of 500 ml divided equally over 24 hours. A calibrated perfusion pump was used to ensure adequate administration.

Blood samples were taken preoperatively, after completion of flap dissection, 1 hour after reperfusion, 24 hours after reperfusion, and on the fourth postoperative day. Blood samples were placed immediately on ice and processed as soon as possible (<5 minutes). Blood was centrifuged at 4°C at 4000 rpm at least within 1 hour after sampling. After centrifugation, 500 μl of plasma was deproteinized using 20 mg of dry sulfosalicylic acid, vortexed, and frozen in liquid nitrogen. Samples were stored at −80°C until analysis. A spectrum of 21 amino acids was determined using fully automated high-performance liquid chromatography described in previous studies.[22]

Primary outcome was the incidence of partial flap loss, subdivided into minor partial flap loss and major partial flap loss. Photographs were taken daily with a ruler within the photograph to determine the area of partial flap loss. The partial flap loss was calculated by planimetry performed with the ImageJ (National Institutes of Health, Bethesda, Md.) program. Minor partial flap loss was managed conservatively and had no adverse effect on outcome (Fig. 1). Major partial flap loss was débrided in the operating room and had a minor effect on aesthetic outcome (Fig. 2). Definitions and abbreviations used for flap complications and their clinical implication are listed in Table 2.

Laser Doppler flowmetry is an accurate method with which to assess localized microcirculation[20,23] and was used to measure flap microcirculatory perfusion, which was measured using laser Doppler flowmetry with hooked probes with the Perimed (Järfälla, Sweden) device in zones I and IV after flap dissection and hourly during the first 5 hours after reperfusion (Fig. 3). Also taken into analysis were flap temperature, perioperative hemodynamics, and previously described risk factors.[24]

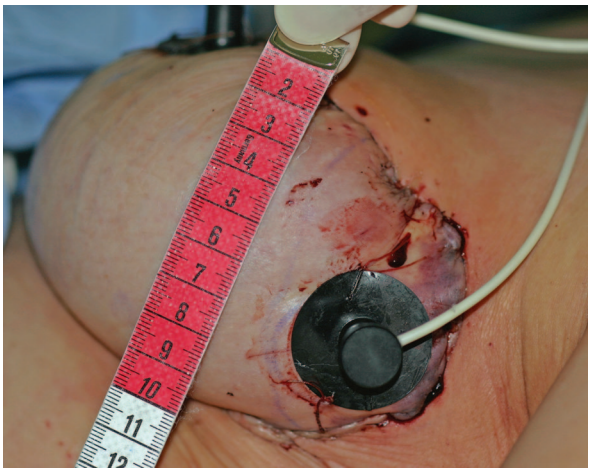

Fig. 1. A patient with minor partial flap loss is shown. Photographs were taken daily with a ruler within the photograph to determine the area of partial flap loss. Minor partial flap loss was managed conservatively and had no adverse effect on outcome.

All data are presented as mean values ± SEM. The patient allocation was revealed to the researchers once recruitment, data collection, and laboratory analyses were complete and incorporated into the SPSS program (SPSS, Inc., Chicago, Ill.), which was then used for statistical analysis. Repeated measures analysis of variance was used for analysis of all repeated measurements. The Wilcoxon-Mann-Whitney test was used for analyzing numerical data, and Fisher's exact test was used for categorical data. A value of $p < 0.05$ (two-tailed) was considered statistically significant.

RESULTS

Twenty patients were included in the study. Two patients had total flap loss caused by anastomosis failure. The cause of this anastomosis failure was attributed to technical failure of the anastomosis rather than intervention-related complication, and therefore data from these patients were excluded from statistical analysis. In the remaining 18 patients, there was a follow-up period of at least 6 weeks after the procedure, sufficient to detect postoperative complications.

Mean age at the time of surgery was 48 ± 1 years and mean body mass index was 24 ± 1. After adaptation of the abdominal flap, the mean weight of flap used for breast reconstruction was 751 ± 47 g. The location of measurement sites in zone I and zone IV remained accessible for measurement in the recon-

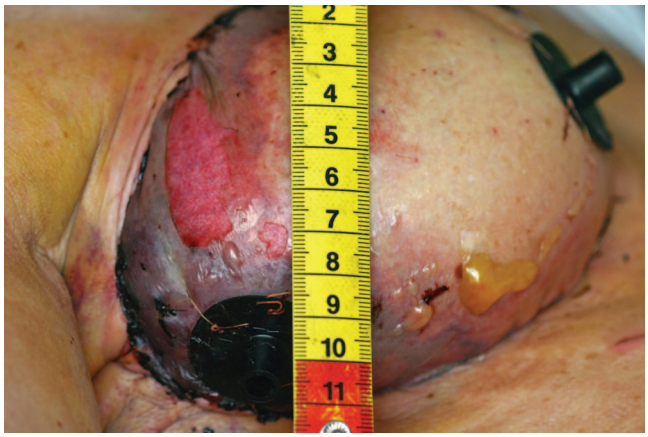

Fig. 2. A patient with major partial flap loss is shown. This was managed with débridement and/or reduction in the reconstructed breast to close the defect and achieve a good aesthetic outcome. Usually, a reduction on the contralateral side was necessary to achieve adequate symmetry.

Table 2. Definitions and Abbreviations Used for Flap Complications

Complication	Abbreviation	Definition	Clinical Impact
Minor partial flap loss	Minor PFL	Loss of a cutaneous portion of the flap with or without fat necrosis; skin necrosis is <5% of the skin surface used in the reconstructed breast	Treated conservatively without adverse outcome; if necessary, scars will be addressed during standard second-stage revision
Major partial flap loss	Major PFL	Same as minor PFL but with >5% loss of skin surface	Return to operating room for débridement; minor effect on aesthetic outcome

PFL, partial flap loss.

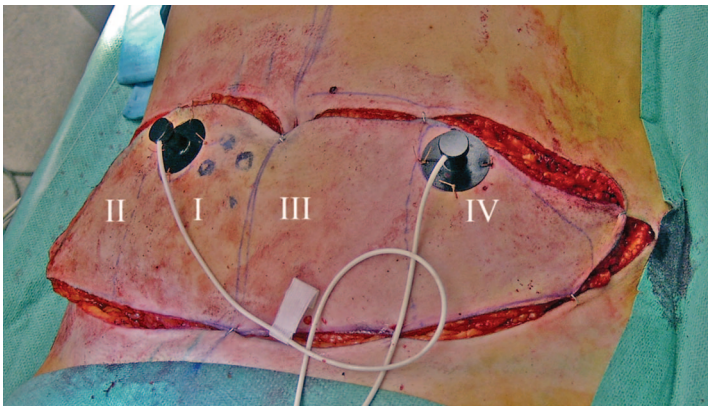

Fig. 3. Perfusion zones are shown during laser Doppler flow measurement after complete dissection of the flap during the preischemia period. Laser Doppler flowmetry was measured simultaneously in zone I and zone IV with the flap still connected to the vascular pedicle.

structed breasts in both groups. Mean flap ischemia time was 52 ± 4 minutes. The prevalence of other risk factors such as radiotherapy and chemotherapy was distributed evenly between the arginine group and the alanine group (Table 1) and did not have an effect on clinical outcome.

From 18 patients, seven (30 percent) experienced partial flap loss, which was always located in zone III or IV (Table 3). Two of these patients were located in the arginine group, compared with five patients in the alanine group. Three patients (17 percent) had major partial flap loss and were all

Table 3. Flap Complications

	All Patients (%)	Arginine (%)	Alanine (%)	*p**
No. of patients	18	8	10	
Flap complications				
All complications	7 (30)	2 (25)	5 (50)	0.27
Major PFL	3 (17)	0 (0)	3 (30)	0.14
Minor PFL	4 (22)	2 (25)	2 (20)	0.62
No complications	11 (61)	6 (75)	5 (50)	0.27

PFL, partial flap loss.
*Fisher's exact test (two-tailed).

in the alanine group; however, this was not significant (Fisher's exact test, $p = 0.15$). These patients required surgical intervention with débridement and/or reduction in the reconstructed breast to close the defect and achieve a good aesthetic outcome. Four patients (22 percent) had minor partial flap loss. This was distributed evenly in both groups. These patients were treated conservatively without adverse outcome. If necessary, scars were corrected during standard second-stage revision.

The concentration of arginine and alanine remained statistically higher in their corresponding groups (Figs. 4 and 5). The arginine metabolites were also higher in the arginine group compared with the alanine group.

No significant differences were detected in the hemodynamics (mean arterial pressure and heart rate) and flap temperature during the first 24 hours when comparing the study groups. In zone I, there was no difference in blood flow between the arginine group and the placebo group. As described previously, laser Doppler flowmetry demonstrated different flow trends in zone I compared with zone IV, indicating a delayed opening and/or vasodilatation of vessels connecting zone I and zone IV of the flap.[20] In the arginine group, zone IV had a higher ($p = 0.04$) microcirculatory blood flow than the alanine control group (Fig. 6).

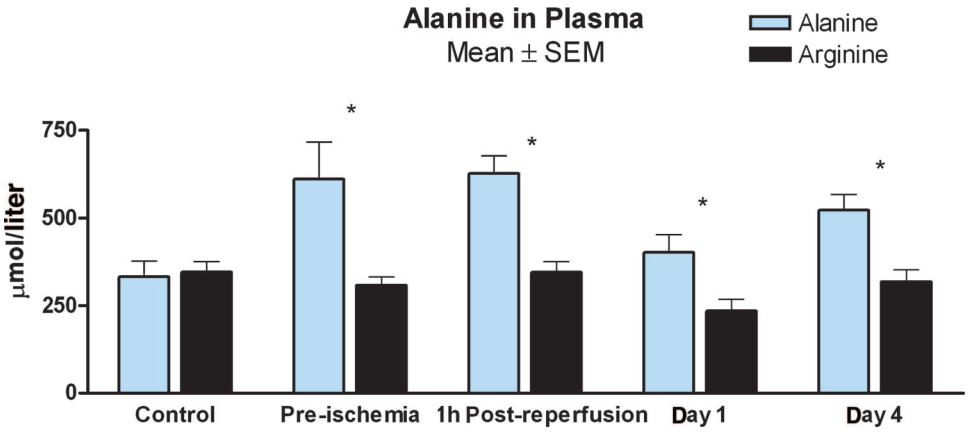

Fig. 4. Alanine plasma concentrations during the perioperative period are displayed. Wilcoxon Mann-Whitney test (two-tailed), $p < 0.05$.

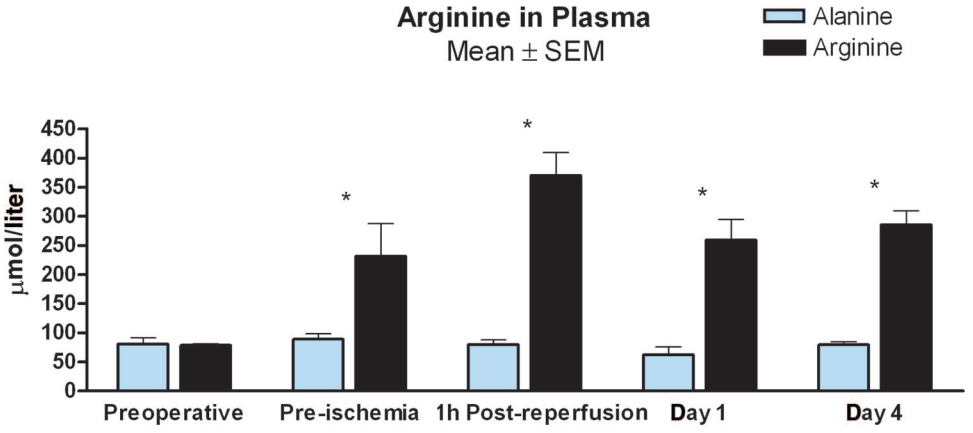

Fig. 5. Arginine plasma concentrations are displayed. Wilcoxon Mann-Whitney test (two-tailed), $p < 0.05$.

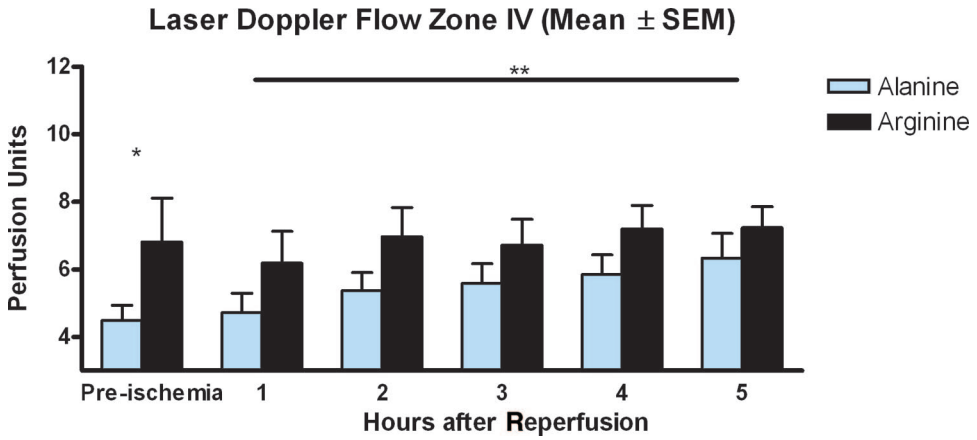

Fig. 6. Laser Doppler flow as measured in zone IV during the perioperative period. *Mann-Whitney *U* test (two-tailed), $p < 0.06$. **Repeated measures analysis of variance (two-tailed), $p < 0.04$.

DISCUSSION

Arginine supplementation significantly increased blood flow in zone IV, the most distal part with less collateral circulation and usually the area at risk for insufficient perfusion and subsequent flap loss. Partial flap loss was observed mainly in the placebo group, and flap complications were more severe.

Use of the Model

Recent studies have demonstrated that despite a high success rate in free flap surgery, complications still are quite common.[25] Even with removal of zone III and zone IV as in bilateral deep inferior epigastric artery perforator (DIEP) flap reconstruction, partial flap loss and/or fat necrosis still occurs. In addition, fat necrosis can lead to a palpable lump that may mimic breast cancer recurrence. This causes extra emotional burden and may lead to secondary corrective surgery. Even in zone I, which is the best perfused zone, fat necrosis can occur.[26] Therefore, studies to further improve these results are needed.

Possible Flaws in the Model

Most recent literature attributes fat necrosis and flap loss to merely venous congestion in zone IV.[27,28] There is a variety in perforator diameter, midline crossover, and deep/superficial venous communications. Therefore, in some cases, the selected perforating vein may not adequately drain the flap and may lead to fat necrosis and/or flap loss.[29,30] However, discarding zone IV does not eliminate the chance of flap complications. Additional venous anastomosis to decrease venous congestion has not been shown to decrease fat necrosis thus far.[27] This suggests that fat necrosis has a multifactor cause and that ischemia-reperfusion may be negated as a cause in clinical studies so far. The net result of venous stasis is an increase of the ischemia period in distal parts of the flap. After opening the choke vessels, the following ischemia-reperfusion injury is more severe. The effect of various strategies for reducing ischemia-reperfusion injury and (partial) flap loss with experimental models in the literature supports this theory.

How to Reduce Reperfusion Injury

Reperfusion injury has wide clinical relevance. It influences the outcome, not solely in free flap (autologous) transplantation in reconstructive surgery, but also in patients with myocardial infarction, stroke, organ transplantation, cardiovascular surgery, and reimplantation of limbs or digits. Despite small clinical studies showing variable success in the treatment of reperfusion injury, there is presently no well-validated intervention that reduces the effect of ischemia-reperfusion injury.[31,32]

Therefore, there have been a large number of experimental studies using different strategies to reduce ischemia-reperfusion injury. The main subjects of these studies were aimed at reducing reactive oxygen species, neutrophil influx, depletion of nitric oxide, and necrosis/apoptosis.[7,8,33] It is widely accepted that the arginine–nitric oxide pathway plays a pivotal role in the pathophysiology of ischemia-reperfusion injury. Supplementation of either the precursor arginine or its end product nitric oxide has given strong evidence for its protective effect in reperfusion injury in experimental studies.[34–36] In addition, modulating the relevant nitric oxide synthase enzymes in experimental studies has given further confirmation.[15,37,38] A recent study

showed that arginine protects from endothelial dysfunction in a human reperfusion model.[39]

Future Models

Because of the relatively high rate of partial flap loss in the placebo group that most likely is caused by the inclusion of zone IV, subsequent studies should discard the use of zone IV. An alternative model could be the DIEP flap, which has less donor-site morbidity. However, with a reduction of perforators, there is increased risk of partial flap loss.[30] Despite discarding zone IV, the DIEP flap is still vulnerable to minor partial flap loss, as demonstrated in clinical studies using ultrasound.[25]

Another strategy may be to combine several strategies to have a cumulative or synergistic effect. Nanobashvili et al. demonstrated that a combined treatment with arginine and antioxidants is superior for reducing ischemia-reperfusion injury when compared with single regiment by means of either arginine or antioxidants.[40] Another easily performed and cheap intervention that can be performed in the clinical setting is preconditioning.[41]

Is Arginine a Good Strategy for Reducing Injury?

This is the first study using arginine in a clinical setting after free tissue transfer. Arginine is a cheap, safe, and easily administrated intervention strategy. We believe that our study gives enough support to start larger studies with arginine intervention in this type of procedure.

CONCLUSIONS

Clinical outcome favors arginine supplementation and leads to an increased microcirculatory blood flow in zone IV. Because of its wide clinical relevance in reconstructive and revascularization procedures, it is important that clinical trials are performed. Reconstructive surgery may be an ideal reperfusion model because of its accessibility for measurement.

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