Perioperative glutamine supplementation restores disturbed renal arginine synthesis after open aortic surgery: a randomized controlled clinical trial

Saskia J. H. Brinkmann,1,* Nikki Buijs,1* Machteld A. R. Vermeulen,2 Efraim Oosterink,6 Henk Schierbeek,6 Albertus Beishuizen,3,4,7 Jean-Paul M. de Vries,5 Willem Wisselink,1 and Paul A. M. van Leeuwen1

1Department of Surgery, VU University Medical Center, Amsterdam, The Netherlands; 2Department of Internal Medicine, VU University Medical Center, Amsterdam, The Netherlands; 3Department of Intensive Care, VU University Medical Center, Amsterdam, The Netherlands; 4Department of Surgery, Medical Centre Alkmaar, Trial Center Holland Health, Alkmaar, The Netherlands; 5Department of Vascular Surgery, St. Antonius Hospital, Nieuwegein, The Netherlands; 6Department of Pediatrics, Academic Medical Center, Emma Children’s Hospital, Amsterdam, The Netherlands; and 7Department of Intensive Care, Medisch Spectrum Twente, Enschede, The Netherlands

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Brinkmann SJ, Buijs N, Vermeulen MA, Oosterink E, Schierbeek H, Beishuizen A, de Vries JP, Wisselink W, van Leeuwen PA. Perioperative glutamine supplementation restores disturbed renal arginine synthesis after open aortic surgery: a randomized controlled clinical trial. Am J Physiol Renal Physiol 311: F567–F575, 2016. First published May 18, 2016; doi:10.1152/ajprenal.00340.2015.—Postoperative renal failure is a common complication after open repair of an abdominal aortic aneurysm. The amino acid arginine is formed in the kidneys from its precursor citrulline, and citrulline is formed from glutamine in the intestines. Arginine enhances the function of the immune and cardiovascular systems, which is important for recovery after surgery. We hypothesized that renal arginine production is diminished after ischemia-reperfusion injury caused by clamping of the aorta during open abdominal aortic surgery and that parenteral glutamine supplementation might compensate for this impaired arginine synthesis. This open-label clinical trial randomized patients who underwent clamping of the aorta during open abdominal aortic surgery to receive a perioperative supplement of intravenous alanlyglmutamine (0.5 g·kg⁻¹·day⁻¹; group B, n = 5) or no supplement (group A, n = 5). One day after surgery, stable isotopes and tracer methods were used to analyze the metabolism and conversion of glutamine, citrulline, and arginine. Whole body plasma flux of glutamine, citrulline, and arginine was significantly higher in group A than in group B (glutamine: 391 ± 34 vs. 258 ± 19 μmol·kg⁻¹·h⁻¹, citrulline: 5.7 ± 0.4 vs. 2.8 ± 0.4 μmol·kg⁻¹·h⁻¹, and arginine: 50 ± 4 vs. 26 ± 2 μmol·kg⁻¹·h⁻¹, P < 0.01), as was the synthesis of citrulline from glutamine (4.8 ± 0.7 vs. 1.6 ± 0.3 μmol·kg⁻¹·h⁻¹), citrulline from arginine (2.3 ± 0.3 vs. 0.96 ± 0.1 μmol·kg⁻¹·h⁻¹), and arginine from glutamine (7.7 ± 0.4 vs. 2.8 ± 0.2 μmol·kg⁻¹·h⁻¹), respectively (P < 0.001 for all). In conclusion, the production of citrulline and arginine is severely reduced after clamping during aortic surgery. This study shows that an intravenous supplement of glutamine increases the production of citrulline and arginine and compensates for the inhibitory effect of ischemia-reperfusion injury.

acute kidney injury; ischemia-reperfusion injury; glutamine; citrulline; arginine

PATIENTS with an abdominal aortic aneurysm are treated by placement of a prosthetic graft in the aorta to prevent rupture of the aneurysm. One of the procedural options in patients with a juxtarenal aneurysm (aneurysms that involve the infrarenal abdominal aorta adjacent to or including the lower margin of the renal artery origins) is an open aortic aneurysm repair. During this procedure, the aorta is clamped above, below, or between the renal arteries, impairing adequate renal perfusion and leading to ischemia-reperfusion injury.

Acute postoperative renal failure is a common complication after open repair of an abdominal aortic aneurysm (occurrence of 6–7%), which may lead to multiorgan failure and higher 30-day mortality. Up to 3.3% of the patients require dialysis, which results in a lower quality of life and higher health care costs (18, 21). Renal failure may result in a diminished renal production of the amino acid arginine from citrulline and could have consequences for the whole body availability of arginine. Nijveldt and colleagues (40, 48) demonstrated in rats and humans that arginine levels dropped during abdominal aneurysm repair, possibly as a consequence of ischemia-reperfusion injury of the kidney.

Arginine is the precursor of the vasodilator nitric oxide (NO) (38, 62), which acts as a mediator in host immune defense and mediates protective effects of the endothelium in the cardiovascular system by acting as a vasodilator (9, 39). Glutamine is a substrate for the de novo production of arginine using the “glutamine-citrulline-arginine pathway.” The synthesis of arginine from glutamine occurs via this intestinal-renal axis (12, 27, 54). Glutamine is converted into citrulline in the intestines, after which citrulline is transported to the kidneys, where arginine production takes place (34, 47).

Recently, arginine and glutamine supplementation have received significant attention because of the potential positive effects on morbidity, wound healing, hospital length of stay, and mortality when given perioperatively (5, 31, 38, 58, 62). When patients are critically ill, however, arginine supplementation may result in a negative outcome, probably due to excessive formation of NO and oxidative metabolites that subsequently leads to oxidative stress (3, 50).

Glutamine supplementation is suggested to be a safe, more physiological way of correcting arginine levels and subsequently achieving glutamine and arginine benefits (20, 60, 66). This was confirmed by results showing that a supplement of glutamine enhanced de novo synthesis of arginine during surgery (3) and that glutamine supplementation was protective for ischemia-reperfusion injury in the intestine and liver and in
renal function in rats (24, 36, 65). Esposito et al. (14) observed that glutamine reduces renal dysfunction and tissue injury associated with ischemia-reperfusion injury in the kidney. Glutamine also improves immunological status, shortens hospital stay after elective abdominal aortic reconstruction, and has positive effects on clinical outcome in several other diseases (2, 23, 26, 31, 41). Vermuelen et al. (56) suggested that a major part of the effects of glutamine can be attributed to the formation of arginine from the administered glutamine. The safety of glutamine in critically ill patients is a topic of an ongoing debate; however, most of the patients in whom glutamine is potentially not safe are septic, mechanically ventilated, and have multiorgan failure (7, 19), which does not apply to the patients in our study.

Only a few studies have used stable isotope techniques to determine the qualitative and quantitative metabolic pathways of glutamine conversion to citrulline and arginine in adults (22, 27–29, 53, 54). Previous studies have shown that almost all citrulline in rodents and humans is derived from the intestinal conversion of glutamine and that the kidneys take up citrulline for de novo synthesis of arginine (12, 27, 54).

This study was conducted to quantitatively demonstrate that the production of arginine is diminished after clamping during aortic surgery and that a parenteral supplement of glutamine, provided as a dipeptide, stimulates citrulline formation and enhances de novo synthesis of arginine in humans after a period of renal ischemia. We therefore designed this stable isotope study with glutamine-supplemented patients and a control group to quantify arginine production from its precursor glutamine after a period of renal ischemia and to establish the contribution of intravenous supplementation of glutamine dipeptide to the synthesis of arginine.

**METHODS**

**Patients.** This open-label randomized controlled trial included 10 patients. An independent clinician used numbered envelopes to randomly assign patients to receive a 0.5 g·kg⁻¹·day⁻¹ alanly-glutamine supplement or no supplement. Patients underwent open surgical repair of an abdominal aortic aneurysm at the VU University Medical Center (Amsterdam, The Netherlands) or at St. Antonius Hospital (Nieuwegein, The Netherlands).

Patients aged 35–75 yr were considered eligible for the study. Exclusion criteria were preexisting kidney and liver failure, pregnancy, use of corticosteroids within 4 wk before surgery, insulin-dependent diabetes mellitus, celiac or Crohn disease, or another major cause of intestinal malabsorption and malnourishment. Patients with a body mass index of <20 kg/m² were considered to be at risk for malnourishment (13).

Liver enzymes and renal function were assessed before the start of the study because both organs are involved in the metabolism of glutamine, citrulline, and arginine. Liver failure was defined as bilirubin levels of >100 μmol/l and kidney failure as an increase in serum creatinine levels to >100 μmol/l. Patients were admitted to the hospital 1 day before surgery. Patient characteristics are shown in Table 1.

Informed consent was obtained from all patients before inclusion. The study protocol was approved by the ethical committees of the VU University Medical Center and St. Antonius Hospital and registered in the trial register (study ID number: NTR2914).

**Study design.** A preoperative blood sample was drawn to measure the baseline concentration of amino acids (glutamine, citrulline, and arginine) and for routine preoperative laboratory tests. Patients were not allowed to eat or drink after 8:00 PM on the evening before surgery until postoperative day 1 of the study. Patients were allocated to one of the following groups: group A or group B. Group A received a continuous intravenous infusion of 0.5 g·kg⁻¹·day⁻¹ alanly-glutamine (Dipeptiven, Fresenius Kabi, Bad Homburg, Germany) that was initiated the evening before surgery and continued until the end of the tracer infusion the day after surgery. Figure 1 shows a schematic overview of the study. The infusion was primed and started before the operation to obtain a balance between the glutamine infusion and whole body flux and to not suddenly increase and change the glutamine pool during the tracer protocol. The infusion was given until after the operation to reach a therapeutic effect. Group B received no supplement.

An intravenous catheter was placed in an antecubital vein and an arterial catheter in the radial artery as part of standard perioperative care. The arterial catheter was used to draw blood samples. Anesthesia and epidural analgesia were applied preoperatively in all patients according to the standard protocol.

Surgery was performed by three experienced surgeons. The operation started the day before the tracer protocol, through a midline incision. The intestines were moved aside to free the aorta. The aorta was exposed and clamped proximal from the aneurysm, suprarenally (n = 5) or infrarenally (n = 5), for a period of time (see RESULTS and Table 1). The aneurysm was incised, and a prosthetic graft was placed in the aorta. The clamp was removed from the aorta to restore the distal blood flow, resulting in reperfusion of both kidneys. The operation was completed according to guidelines and protocol, and patients were transferred to the intensive care unit.

**Stable isotope tracers.** The stable isotope methodology has been thoroughly explained in the book by Wolfe et al. (61) and in previous studies by our group (6, 27–29). In brief, stable isotope tracers behave the same as the amino acids of interest, but one atom in the tracer is replaced with an isotope that has an extra neutron. The tracer quantifies the endogenous presence of an amino acid and the conversion rate of one amino acid into another at the whole body level. The tracers L-[2-¹⁵N]glutamine, L-[5-¹⁵N]C-4,4,4,5,5H₅]citrulline, and L-[¹⁵N]arginine were purchased from Cambridge Isotope Laboratories (Woburn, MA) and are from this point noted as glutamine [M⁺1], citrulline [M⁺5], and arginine [M⁺2], respectively.

The Department of Clinical Pharmacy at Erasmus Medical Center (Rotterdam, The Netherlands) prepared sterile and pyrogen-free stock solutions of the tracers. The glutamine tracer was prepared the day before surgery, owing to the limited stability of soluble glutamine of only 72 h. Stock solutions were diluted with normal saline just before the start of the tracer infusion. The tracer dosages for citrulline [M⁺5] and arginine [M⁺2] were comparable with other studies, and the glutamine [M⁺1] tracer dosage was corrected for the simultaneous alanly-glutamine infusion to reach adequate enrichments (Table 2) (6, 27, 28, 53).

**Tracer infusion, blood sampling, and measurements.** The tracer protocol started after 24 h after the first operative incision. A baseline blood sample was first drawn from the radial artery catheter, and a calibrated, volume-controlled pump (Graseby 3000, Graseby Medical, Watford, UK) was used to administer a primed-continuous infusion of the stable isotopes through the intravenous catheter. The tracer was prepared the day before surgery, owing to the limited stability of soluble glutamine of only 72 h. Stock solutions were diluted with normal saline just before the start of the tracer infusion. The tracer dosages for citrulline [M⁺5] and arginine [M⁺2] were comparable with other studies, and the glutamine [M⁺1] tracer dosage was corrected for the simultaneous alanly-glutamine infusion to reach adequate enrichments (Table 2) (6, 27, 28, 53).

**Blood sampling and analysis.** HPLC was used to measure amino acid concentrations in deproteinized samples and infusates, as previously described (55). Blood was collected in heparinized vacuum tubes (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ) and placed on ice. First, the hematocrit of the blood samples was measured. Blood was centrifuged (10 min, 3,000 rpm, 4°C), and 50 μl of the supernatant were deproteinized with 20 mg dry sulfosalicylic acid within 1 h after sampling. After being mixed, samples were stored at −80°C until analysis.

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Mass spectrometric analysis. Plasma enrichments of the infused tracers and the tracer products were measured by liquid chromatography-mass spectrometry (LC/MS) according to a recently developed novel method (44). Briefly, 20 μL hydrochloric acid, 20 μL internal standard, and 200 μL cation exchange solution [0.1 mg/mL AG 50W-X8 resin (Bio-Rad), 200–400 mesh] were added to 50 μL deproteinized plasma. After being mixing and centrifuged, the supernatant was removed. The resin was washed with 1 mL water, and amino acids were extracted twice with 500 and 200 μL of 6 mol/L ammonia, respectively. The eluate was dried in a SpeedVac (Genevac) and redissolved in 80 μL acetonitrile, 20 μL of 1 mol/L sodium carbonate, 200 μL water, and 140 μL pyridine-ethanol (4:1).

Derivatives of the amino acids were prepared by adding ethyl chloroformate and incubating for 5 min. After two extractions with ethyl acetate, the combined solutions of the first (400 μL) and second (400 μL) extractions were evaporated under a gentle stream of nitrogen at room temperature until almost dry and redissolved in 100 μL of 20% methanol. Analyses were performed with LC/MS (Velos Pro, Thermo Fisher) by injecting 10 μL of the sample extract on a 2.1- to 100-mm, 1.7-mm Waters Acquity BEH C18 column. Elution was

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A (Alanyl-Glutamine Supplementation)</th>
<th>Group B (Controls)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients/group</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>67 ± 3</td>
<td>72 ± 3</td>
<td>0.28</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74 ± 9</td>
<td>82 ± 7</td>
<td>0.73</td>
</tr>
<tr>
<td>Height, cm</td>
<td>175 ± 5</td>
<td>175 ± 5</td>
<td>0.43</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23 ± 2</td>
<td>27 ± 2</td>
<td>0.37</td>
</tr>
<tr>
<td>Amino acid concentration, μmol/l (time of inclusion, before operation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>880 ± 32</td>
<td>773 ± 34</td>
<td>0.06</td>
</tr>
<tr>
<td>Citrulline</td>
<td>35 ± 4</td>
<td>32 ± 2</td>
<td>0.57</td>
</tr>
<tr>
<td>Arginine</td>
<td>86 ± 5</td>
<td>82 ± 11</td>
<td>0.77</td>
</tr>
<tr>
<td>Hours from surgery to start tracer protocol</td>
<td>23 ± 1</td>
<td>24 ± 1</td>
<td></td>
</tr>
<tr>
<td>Amino acid concentration, μmol/l (T02)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>746 ± 48*</td>
<td>465 ± 16*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Citrulline</td>
<td>26 ± 2*</td>
<td>14 ± 1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arginine</td>
<td>40 ± 4*</td>
<td>30 (23–33)*</td>
<td>0.05</td>
</tr>
<tr>
<td>Preoperative laboratory results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>93 ± 20</td>
<td>93 (72–139)</td>
<td>0.99</td>
</tr>
<tr>
<td>Urea, mmol/l</td>
<td>6 ± 1</td>
<td>7 ± 1</td>
<td>0.82</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate, ml·min⁻¹·1,73 m²</td>
<td>54 (43–60)</td>
<td>62 ± 6</td>
<td>0.33</td>
</tr>
<tr>
<td>Bilirubin, μmol/l</td>
<td>8 ± 2</td>
<td>6 ± 1</td>
<td>0.37</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>27 ± 2</td>
<td>22 ± 3</td>
<td>0.22</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>6 ± 0.2</td>
<td>6 ± 0.4</td>
<td>0.43</td>
</tr>
<tr>
<td>Postoperative laboratory results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>114 ± 23*</td>
<td>156 ± 30*</td>
<td>0.30</td>
</tr>
<tr>
<td>Urea, mmol/l</td>
<td>11 ± 2*</td>
<td>11 ± 1*</td>
<td>0.80</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate, ml·min⁻¹·1,73 m²</td>
<td>50 (33–60)*</td>
<td>33 ± 4*</td>
<td>0.22</td>
</tr>
<tr>
<td>Clamping method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suprarenal</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infraarenal</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clamping time, min</td>
<td>31 ± 3</td>
<td>60 (24–110)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total blood loss during surgery, ml</td>
<td>760 ± 136</td>
<td>1110 ± 165</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE and medians ± interquartile ranges, respectively; T0, the day after operation after administration of intravenous 0.5 g·kg⁻¹·h⁻¹ alanyl-glutamine (only in group A) just before the start of the tracer infusion. An independent-sample t-test or Mann-Whitney U-test was used to determine significant differences (P < 0.05). *Significant difference between pre- and postoperative values.

Mass spectrometric analysis. Plasma enrichments of the infused tracers and the tracer products were measured by liquid chromatography-mass spectrometry (LC/MS) according to a recently developed novel method (44). Briefly, 20 μL hydrochloric acid, 20 μL internal standard, and 200 μL cation exchange solution [0.1 mg/mL AG 50W-X8 resin (Bio-Rad), 200–400 mesh] were added to 50 μL deproteinized plasma. After being mixing and centrifuged, the supernatant was removed. The resin was washed with 1 mL water, and amino acids were extracted twice with 500 and 200 μL of 6 mol/L ammonia, respectively. The eluate was dried in a SpeedVac (Genevac) and redissolved in 80 μL acetonitrile, 20 μL of 1 mol/L sodium carbonate, 200 μL water, and 140 μL pyridine-ethanol (4:1).

Derivatives of the amino acids were prepared by adding ethyl chloroformate and incubating for 5 min. After two extractions with ethyl acetate, the combined solutions of the first (400 μL) and second (400 μL) extractions were evaporated under a gentle stream of nitrogen at room temperature until almost dry and redissolved in 100 μL of 20% methanol. Analyses were performed with LC/MS (Velos Pro, Thermo Fisher) by injecting 10 μL of the sample extract on a 2.1- to 100-mm, 1.7-mm Waters Acquity BEH C18 column. Elution was
performed at a stable temperature of 40°C using mobile phases consisting of 0.5 mmol/l tridecafluoroheptanoic acid and 1 mmol/l formic acid (phase A) and methanol with 0.5 mmol/l tridecafluoroheptanoic acid and 1 mmol/l formic acid (phase B). Ion abundance was monitored in full scan using the zoom-scan mode for glutamine [mass-to-charge ratio (m/z): 245–260], citrulline (m/z: 274–290), ornithine (m/z: 303–320), and arginine (m/z: 273–290) (6).

Calculations. The equations used for calculating the whole body metabolism of glutamine, citrulline, and arginine are shown in Table 3. Enrichment curves were fitted to determine a mean steady-state value on the different time points per individual using Prism 5.0 software (GraphPad Software, La Jolla, CA). For all patients, we found a steady state after 60 min of infusion for glutamine, citrulline, and arginine. Isotope enrichments were calculated as enrichment at steady state minus isotopic background measurements at baseline and are expressed as mol percent excess. Whole body fluxes of glutamine, citrulline, and arginine were calculated, and whole body conversion rates for glutamine into citrulline and citrulline into arginine were determined.

Statistical analysis. Previous studies have shown that a small number of patients is sufficient to observe significant conversions of glutamine into citrulline and arginine at the whole body level (6, 27–29). A sample size of 5 patients/group was thus calculated to provide a power of 1.0 (α ≤ 0.05) to determine a difference between the intervention and control groups (Program PS: Power and Sample Size Calculation, version 2.1.31, 2004, by Dupont and Plummer, Department of Biostatistics, Vanderbilt University). The Shapiro-Wilk normality test and Q-Q plots were used to test results at distribution. Normally distributed data are expressed as means ± SE. Non-normally distributed data are expressed as medians and interquartile ranges (IQRs). An independent sample t-test or a Mann-Whitney U-test was used to compare the control group with the alanyl-glutamine group, according to distribution. A one-sample t-test was used to test whether the steady state of metabolic products differed from zero. A two-tailed P value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS 17.0 software (IBM, Armonk, NY).

RESULTS

Patients. Ten patients were included between June 2011 and September 2012: five patients (group A) received 0.5 g·kg⁻¹·day⁻¹ alanyl-glutamine for at least 42 h before the start of the tracer protocol and five control patients (group B) did not receive a supplement. Baseline characteristics of the patients are shown in Table 1. The body mass index of patients did not reveal malnourishment. Compared with preoperative values, amino acid concentrations and fluxes showed linearity, indicating that the distribution was consistent with the assumption of normality.

Alanyl-glutamine infusion. Patients in group A received an average of 37 ± 5 g of alanyl-glutamine per day. This resulted in approximate amounts of 19 ± 4.0 g in the 18 h before surgery at day 0, 43 ± 0 g in the 42 h before the tracer protocol at day +1, and 3.9 ± 0.5 g during the 2.5-h tracer infusion. Alanyl-glutamine was also infused during the tracer protocol to keep glutamine metabolism with the supplement in steady state.

Amino acid concentrations. Before surgery, baseline plasma concentrations of glutamine, citrulline, and arginine concentrations did not differ between the groups. After surgery, plasma concentrations decreased significantly in both groups compared with baseline (P < 0.05; see Table 1). Plasma concentrations of glutamine, citrulline, and arginine were significantly higher postoperatively in group A than in group B (P < 0.05).

Enrichments of isotopes. We found significant enrichments of the infused isotopes of glutamine [M +1], citrulline [M +5], and arginine [M +2] in both groups (P < 0.001), with a slight overshoot at the beginning of the steady-state plateau due to a small overestimated amount of the priming doses. Their metabolic products citrulline [M +1], arginine [M +1], and arginine [M +5] were significantly different from zero (P < 0.001), indicating that the distribution was consistent with the assumption of normality.

Table 3. Equations

<table>
<thead>
<tr>
<th>Equation</th>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E, enrichment in the infusate; Eᵢ, enrichments in plasma at steady state; Qᵢ, whole body conversion rate during alanyl-glutamine infusion</td>
<td>Qᵢ = i(Eᵢ/Eᵢ) − 1</td>
<td></td>
</tr>
<tr>
<td>Qᵢ, whole body glutamine turnover</td>
<td>Qᵢ = Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ</td>
<td></td>
</tr>
<tr>
<td>Qᵢ, whole body glutamine-to-citrulline conversion rate</td>
<td>Qᵢ = Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ</td>
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</tr>
<tr>
<td>Qᵢ, whole body glutamine-to-arginine conversion rate</td>
<td>Qᵢ = Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ</td>
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<tr>
<td>Qᵢ, whole body glutamine-to-citrulline conversion rate</td>
<td>Qᵢ = Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ + Eᵢ/Mᵢ</td>
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</table>

Table 2. Tracer dosages

<table>
<thead>
<tr>
<th>Group</th>
<th>Priming dose, μmol/kg</th>
<th>Infusion dose, μmol·kg⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>23.8</td>
<td>25.3</td>
</tr>
<tr>
<td>Cit</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Arg</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln</td>
<td>26.8</td>
<td>16.7</td>
</tr>
<tr>
<td>Cit</td>
<td>1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Arg</td>
<td>3.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>
which states that the labels of glutamine [M+1] and citrulline [M+5] are metabolized into citrulline and arginine molecules. Arterial plasma enrichments of the infused tracers and the tracer products were observed to be in steady state for both groups (Fig. 2).

**Whole body amino acid flux.** The whole body plasma flux of glutamine in group A was 487.1 ± 34.3 μmol·kg⁻¹·h⁻¹. When corrected for the alanyl-glutamine infusion of 0.5 g·kg⁻¹·day⁻¹, the endogenous glutamine flux was 391.1 ± 34 μmol·kg⁻¹·h⁻¹. The endogenous flux was calculated by subtracting the glutamine given with this infusion (exogenous flux) from the flux to obtain endogenous flux only. The whole body plasma flux was 5.7 ± 0.4 μmol·kg⁻¹·h⁻¹ for citrulline and 49.6 ± 3.9 μmol·kg⁻¹·h⁻¹ for arginine in group A. These fluxes were significantly higher for all amino acids in group A compared with group B (total glutamine: 258.8 ± 19.2 μmol·kg⁻¹·h⁻¹, citrulline: 2.8 ± 0.4 μmol·kg⁻¹·h⁻¹, and arginine: 25.9 ± 1.7 μmol·kg⁻¹·h⁻¹, P < 0.001; Fig. 3).

**Citrulline production from glutamine.** The estimated whole body citrulline [M+1] synthesis from plasma glutamine [M+1] in group A was 4.8 ± 0.7 μmol·kg⁻¹·h⁻¹, representing 85% of the total citrulline flux. In group B, citrulline [M+1] synthesis was 1.6 ± 0.3 μmol·kg⁻¹·h⁻¹ (56% of whole body citrulline flux), which was significantly less than in group A (P < 0.01; Fig. 4).

**Arginine production from citrulline.** The estimated whole body arginine [M+5] synthesis from plasma citrulline [M+5] in group A was 2.3 ± 0.3 μmol·kg⁻¹·h⁻¹, representing 40% of the total citrulline flux. In group B, 35% of the total citrulline flux was used for arginine production of 0.96 ± 0.1 μmol·kg⁻¹·h⁻¹, which was significantly lower than in group A (P < 0.001; Fig. 4).

**DISCUSSION.** The primary aim of the present study was to demonstrate the detrimental effects of renal ischemia-reperfusion injury on the whole body metabolism of glutamine, citrulline, and arginine in patients in the early postoperative, postabsorptive state and to investigate whether a periopeative supplement of glutamine would prevent this decrease. In line with our hypothesis, we demonstrated, for the first time, that plasma concentrations of glutamine, citrulline, and arginine were significantly decreased after ischemia-reperfusion injury by aortic clamping in vivo. The whole body flux of glutamine, citrulline, and arginine was significantly higher when patients received a supplement of alanly-glutamine. The whole body flux of citrulline and arginine from glutamine was significantly higher in the group supplemented with glutamine than in the control group, and the de novo synthesis of arginine was doubled in patients that received glutamine.
Besides being the precursor of NO and therefore important for the immune and cardiovascular systems, arginine is of great importance for wound healing (11). Arginine levels drop when patients are exposed to surgical injury, cancer, critical illness, or infection, and arginine then becomes an essential amino acid (37, 57). Arginine deficiency may lead to immunosuppression, impaired recovery, a disturbed inflammatory response, and diminished vascular function (37, 62).

Fasting before surgery results in a depletion of glycogen stores, dehydration, muscle wasting, a weakened immune response, unnecessary production of inflammatory mediators, and a drop in glutamine, citrulline, and arginine levels (36). Patients in the present study fasted during the entire study protocol. The metabolic response to surgical stress and critical illness is characterized by muscle protein breakdown and mobilization of amino acids to be used by the immune system, gut, and liver, as reflected in our results that showed a decrease in the plasma concentrations of all amino acids. This is also reflected by the difference in the estimated whole body flux of arginine [M+2] (25.9 ± 1.7 \( \mu \)mol·kg\(^{-1}\)·h\(^{-1}\)) compared with the much lower de novo production of arginine from citrulline (0.96 ± 0.1 \( \mu \)mol·kg\(^{-1}\)·h\(^{-1}\)). The whole body flux is representative for the rate of the appearance and disappearance at the time of measurement in steady state.

When patients undergo an open aortic aneurysm repair, additional ischemia-reperfusion injury occurs in the kidneys. In brief, tissue ischemia occurs during cross-clamping. This causes an anaerobic cellular metabolism, leading to acidosis, as well as decreased cellular glycogen and ATP levels that cause reduced cell membrane function, vascular endothelial adhesions, and an upregulation of clotting pathways (1). The most detrimental part of this cascade is the production of oxygen free radicals when cellular metabolism shifts from anaerobic back to aerobic after clamping. After the restoration of blood flow, these free radicals cause further damage to the ischemic tissue by neutrophil influx and depletion of NO, aggravating interstitial edema, vasoconstriction, the accumulation of toxins, and the production of proinflammatory mediators (25, 33). This ischemia-reperfusion process causes serious damage to tissues and diminishes cell function.

To demonstrate that the pathway of glutamine to arginine is disturbed primarily by ischemia-reperfusion injury and not by the effect of surgery itself, we compared our results with those of a study of similar design by Lighthart-Melis et al. (27) in which stable isotopes were used to quantify the metabolism of glutamine, citrulline, and arginine. They used intravenous stable isotopes in patients in the postabsorptive state during abdominal surgery to determine renal metabolism (27). The whole body glutamine flux in their surgical patients was similar to our control group. These levels were in the normal reference range, as previously described (8, 9, 27). The whole body glutamine flux was 6.2 ± 0.6 \( \mu \)mol·kg\(^{-1}\)·h\(^{-1}\), whereas the flux in our patients was less than half of this (2.8 ± 0.2 \( \mu \)mol·kg\(^{-1}\)·h\(^{-1}\)). The whole body arginine flux appeared to be decreased as well to almost half compared with the results of Lighthart-Melis et al. (42 ± 2.9 vs. 26 ± 1.7 \( \mu \)mol·kg\(^{-1}\)·h\(^{-1}\), respectively). Conversion rates of glutamine to citrulline (5.1 ± 0.7 vs. 1.55 ± 0.3 \( \mu \)mol·kg\(^{-1}\)·h\(^{-1}\)) and citrulline to arginine (4.9 ± 0.9 vs. 0.96 ± 0.11 \( \mu \)mol·kg\(^{-1}\)·h\(^{-1}\)) were seriously reduced after ischemia-reperfusion. Thus, citrulline and arginine fluxes seem to be substantially diminished after ischemia and reperfusion of the kidneys and gut.

The present study shows that the first step in the intestinal-renal axis, the conversion of glutamine to citrulline in the enterocyte, is disturbed after open abdominal aneurysm repair. The intestine is very susceptible to ischemia due to the unique anatomic arrangement of the mucosal microcirculation and labile cells (17, 30). Subsequent reperfusion of the intestine results in the further development of hemorrhagic mucosal damage by oxygen free radicals and activation of phospholipase A\(_2\) (51).

Although the superior mesenteric artery, which supplies the small intestine with oxygen-rich blood, is not directly clamped during elective open aneurysm repair, the intestine is exposed to ischemia secondary to altered blood flow (42). Mesenteric traction and cooling caused by intestinal manipulation exacerbates intestinal ischemia secondary to vasoactive amine release and anatomic distortion of small blood vessels (30). In addition, ischemia, trauma, and intestinal cooling result in an impaired mucosal barrier leading to endotoxin translocation (49). Dysmotility of the small intestine was noted in a large group of surgical patients undergoing abdominal aortic aneurysm repair (29). This leads to impaired absorption of enteral nutrition, suggesting that the small bowel does not function properly after abdominal aneurysm repair (15, 35). Studies have shown that a functional competent enterocyte is required to convert glutamine into citrulline (10, 45, 46). Our results suggest that enterocyte function is indeed disturbed during open aneurysm repair because the synthesis of citrulline is ~70% lower than in other surgical patients (50).

The second step in the intestinal-renal axis takes place in the kidneys. It was demonstrated that the kidneys show (temporary) damage postoperatively, even when the aorta is clamped infrarenally (16, 63), which has direct consequences for the availability of arginine. Prins et al. (48) found decreased renal arginine production in rats after ischemia-reperfusion injury, which could contribute to or slow the recovery from the low plasma levels of arginine as seen during surgery and after clamping. Nijveld et al. (40) showed low arginine plasma levels after aortic surgery in patients exposed to ischemia-reperfusion injury as a result of clamping.
We used stable isotopes to quantitatively demonstrate that ischemia-reperfusion injury results in a decrease in arginine fluxes of 44% compared with patients who were under normal surgical conditions without ischemia-reperfusion injury and of up to 90% compared with healthy individuals (8, 27). The likelihood of kidney damage after clamping in our study group was confirmed by the significantly worse rate of creatinine clearance and estimated glomerular filtration compared with preoperative values (43). However, the number of patients was too small to draw definite conclusions regarding this hypothesis.

To assess whether a perioperative supplement of alanyl-glutamine restores low levels of amino acids after ischemia-reperfusion injury, we compared our results with a study by Buijs et al. (6) in which glutamine, citrulline, and arginine fluxes were determined with stable isotopes peroperatively. We found that the whole body flux of glutamine postoperatively did not differ from their peroperative results (6). Thus, levels of glutamine appeared to be preserved after ischemia-reperfusion injury when a alanyl-glutamine supplement was given peroperatively. The conversion of glutamine to citrulline was 8.1 ± 0.9 μmol·kg⁻¹·h⁻¹ in the peroperative study, which was almost twice as high as in our intervention group postoperatively (4.8 ± 0.7 μmol·kg⁻¹·h⁻¹, P < 0.01). The citrulline-to-arginine conversion seemed to be twice as high compared with our group exposed to renal ischemia-reperfusion injury (4.5 ± 0.60 vs. 2.29 ± 0.31 μmol·kg⁻¹·h⁻¹). This proves that de novo synthesis of citrulline and arginine is diminished even when a supplement of glutamine is given, probably due to damaged enterocytes and kidney damage. However, plasma concentrations of glutamine, citrulline, and arginine were significantly higher in the intervention group. Compared with our control group, this supplement of glutamine is capable of preserving glutamine-to-citrulline fluxes in the intestine and citrulline-to-arginine fluxes in the kidney as well as keeping glutamine levels at perioperative values (P < 0.01).

The results of this study are relevant for clinical practice because they provide more insights into the detrimental effects of ischemia-reperfusion injury and how to overcome these. Studies have shown intravenous glutamine administration is safe (60, 66). In connection with surgery, glutamine administration improves recovery from injury, positively affects survival, and reduces infectious complications and hospital and intensive care unit lengths of stay (3, 64, 66). In addition, ESPEN Guidelines on Enteral Nutrition in Surgery still recommend giving arginine preoperatively in abdominal surgery because of the reduced postoperative morbidity and length of stay (4, 52, 59).

This study has some limitations. First, we did not measure organ fluxes to calculate the specific contribution of the intestine and kidney because this was not possible in postoperative setting. However, Buijs et al. (6), Lighthart-Melis et al. (27–29), and van de Poll et al. (54) have extensively demonstrated the contribution of these two organs to this conversion. Second, different clamping methods were used (suprarenal and infrarenal), which could have influenced the rate and amount of ischemia-reperfusion injury to the kidney. However, ischemia-reperfusion injury to the kidney still occurs with infrarenal clamping (16, 63). When the groups were divided by infrarenal and suprarenal clamping, no significant differences were seen. The difference between groups in clamping time was almost doubled but was not significant. Furthermore, the patients in this study were relatively older and representative of patients with abdominal aortic aneurysm. The body mass index was not significantly different between the groups, but more overweight people were in group B. This might have influenced our metabolic measurements.

Similar to the results of Buijs et al., we also found an overestimate in the quantitative contribution of glutamine to arginine. Debate about the interpretation of the use of this glutamine [M+1]/[2-15N]glutamine tracer is ongoing. We are aware that this tracer is also converted into L-[15N]glutamine, as demonstrated by Marini et al. (32), who showed that the use of a [2-15N]glutamine tracer may overestimate the quantitative contribution of glutamine to arginine in mice due to nitrogen recycling. Tomlinson et al. (53) found, similar to our results and the results of Buijs et al., that the 15N-labeled tracer showed a contribution of glutamine [M+1] to arginine [M+1] synthesis that exceeded the citrulline-to-arginine conversion. However, they also demonstrated that overestimation of arginine production is possible because the labeled 15N-atom was found in various locations of the ornithine, citrulline, and arginine molecules. Our study, however, was designed to determine the first pathway effects of aortic clamping on citrulline and arginine metabolism and whether parenteral glutamine, as a supplement of 0.5 g·kg⁻¹·day⁻¹, could enhance this synthesis; hence, our chosen methods (6, 44). Our aim was not to analyze the metabolism of the different fragments of the molecules of glutamine, citrulline, and arginine. We also did not intend to study the biochemical transitions of nitrogen on the amino acid molecules, because various studies have already showed this precursor pathway in humans using the stable isotope method (6, 27, 53). However, the quantitative results of the 15N-labeled glutamine tracer should be interpreted with caution because outcomes may overestimate the contribution of glutamine to citrulline and arginine formation.

In conclusion, this is the first study to quantify the whole-body fluxes of glutamine, citrulline, and arginine and the conversion of glutamine to citrulline and of citrulline to arginine after ischemia-reperfusion injury of the kidneys. We demonstrate that a perioperative supplement of alanyl-glutamine is capable of preserving whole body and organ fluxes of glutamine, citrulline, and arginine to perioperative values in patients in the postoperative and postabsorptive state exposed to ischemia-reperfusion injury of the kidneys caused by clamping during abdominal aortic surgery.

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