Nutrition before, during, and after surgery increases the arginine:asymmetric dimethylarginine ratio and relates to improved myocardial glucose metabolism: a randomized controlled trial¹⁻³

Marlieke Visser, Mariska Davids, Hein J Verberne, Wouter EM Kok, Robert Tepaske, Riccardo Cocchieri, Elles M Kemper, Tom Teerlink, Marianne A Jonker, Willem Wisselink, Bas AJM de Mol, and Paul AM van Leeuwen

ABSTRACT

Background: Nitric oxide (NO) is essential for the optimal perfusion of the heart and its vasculature. NO may be insufficient in surgical patients because its precursor arginine is decreased, and the inhibitor of NO synthesis asymmetric dimethylarginine (ADMA) is increased. Besides arginine, the presence of other amino acids essential for the proper metabolism of cardiac cells may be decreased too. Supplementation of these amino acids with enteral and parenteral nutrition before, during, and after surgery may augment the myocardial and plasma arginine:ADMA ratio and availability of amino acids. Myocardial glucose metabolism and nutritional conditioning may result in a reduction of cardiac injury and support rapid recovery after major surgery.

Objective: We investigated the effect of nutrition before, during, and after surgery on amino acids and the myocardial arginine:ADMA ratio and its relation to myocardial glucose metabolism.

Design: In this trial, 33 patients who were undergoing off-pump coronary artery bypass grafting (CABG) were randomly assigned between enteral, parenteral, or no nutrition (control) from 2 d before, during, and until 2 d after surgery. Both enteral and parenteral solutions were prepared with commercially available products and included proteins or amino acids, glucose, vitamins, and minerals. Concentrations of amino acids including ADMA were analyzed in myocardial tissue and plasma samples. ¹⁸F-fluorodeoxyglucose positron emission tomography was performed before and after surgery to assess myocardial glucose metabolism.

Results: The myocardial arginine:ADMA ratio increased during surgery and was significantly higher in the control group [median (IQR): 115.0 (98.0–142.2) (P = 0.012), 116.9 (100.3–135.3) (P = 0.004), and 93.3 (82.7–101.1), respectively]. Furthermore, the change in the preoperative to postoperative plasma arginine:ADMA ratio correlated with the change in myocardial glucose metabolism in positron emission tomography (r = 0.427, P = 0.033).

Conclusion: Enteral or parenteral nutrition before, during, and after CABG may positively influence myocardial glucose metabolism by increasing the plasma and myocardial arginine:ADMA ratio. This trial was registered at http://www.trialregister.nl as NTR2183.

INTRODUCTION

Endothelium-derived nitric oxide (NO)⁴ is a prominent compound in the heart that is necessary for the proper functioning of the cardiovascular system (1). NO is synthesized by nitric oxide synthase (NOS) from arginine, which is an amino acid that becomes essential in times of stress as, eg, during surgery and heart failure (2, 3). Increased extracellular arginine can be taken up by endothelial cells and contribute to NO synthesis (4). Besides arginine availability, NO production is influenced by the NOS inhibitor asymmetric dimethylarginine (ADMA), which is a product of protein methylation (4). A high plasma ADMA concentration has been reported in critically ill patients (5), is considered a risk factor for chronic cardiovascular disease, and is an indicator of a worse clinical outcome in patients with diminished cardiac or vascular dysfunction (4). Because ADMA inhibits NO production by competing with arginine for NOS binding, the net production of NO likely depends on the ratio between the substrate and inhibitor (ie, the arginine:ADMA ratio). Indeed, the arginine:ADMA ratio was a better predictor of mortality than arginine or ADMA alone (6) and associated with the severity of chronic heart failure (7).

Besides arginine, several other amino acids are essential for proper cardiac metabolism (8, 9). Amino acids are able to replenish components of the tricarboxylic acid cycle, which can increase ATP production in heart cells, with positive effects on cardiomyocyte metabolism. Many of these amino acids are essential amino acids that cannot be synthesized by the human body.

¹From the Departments of Surgery (MV, WW, and PAMvL), Clinical Chemistry (MD and TT), and Epidemiology and Biostatistics (MAJ), VU University Medical Center, Amsterdam, Netherlands, and the Departments of Cardio-thoracic Surgery (MV, RC, and BAJMdM), Nuclear Medicine (HJV), Cardiology (WEMK), and Pharmacy (EMK) and the Intensive Care Unit (RT), Academic Medical Center University of Amsterdam, Amsterdam, Netherlands.

²No funding was received for this work.

³Address correspondence to PAM van Leeuwen, Department of Surgery, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, Netherlands. E-mail: pam.vleeuwen@vumc.nl.

⁴Abbreviations used: ADMA, asymmetric dimethylarginine; AMC, Academic Medical Center of the University of Amsterdam; BCAA, branched-chain amino acid; CABG, coronary artery bypass grafting; KW, Kruskal-Wallis test; LAD, left anterior descending coronary artery; MW, Mann-Whitney U test; NO, nitric oxide; NOS, nitric oxide synthase; RCA, right coronary artery; RCX, left circumflex coronary artery; SUV, standardized uptake value; ¹⁸F-FDG, ¹⁸F-fluorodeoxyglucose.

Received September 24, 2013. Accepted for publication March 5, 2014. First published online April 2, 2014; doi: 10.3945/ajcn.113.075473.
NUTRITION DURING SURGERY INCREASES ARGinine:ADMA

and, therefore, need to be supplied through nutrition. Furthermore, amino acids, and especially branched-chain amino acids [BCAAs (ie, leucine, isoleucine, and valine)], function as precursors for myocardial protein synthesis (9–11).

Therefore, increasing the arginine:ADMA ratio and concentrations of other amino acids in the heart with nutrition might be beneficial for cardiovascular metabolism (4). In particular, surgical patients might benefit because they have low plasma concentrations of arginine (12) and other amino acids (13), probably because of excessive catabolism and use for wound healing (14). Furthermore, proper myocardial glucose metabolism was associated with a better prognosis in patients who were undergoing revascularization than in those with low glucose metabolism (15). To minimize surgery-induced decreases in myocardial amino acid concentrations, we hypothesized that nutrition should be given before, after, and, especially, during surgery (16). To our knowledge, studies in which enteral and parenteral nutrition before and during surgery increases the myocardial arginine:ADMA ratio and concentrations of BCAAs in these patients. Furthermore, we studied the associations between changes in the preoperative to postoperative plasma arginine:ADMA ratio and changes in preoperative to postoperative myocardial glucose metabolism.

SUBJECTS AND METHODS

Study design and protocol

This randomized, controlled, intervention study was performed at the Department of Cardio-thoracic Surgery at the Academic Medical Center of the University of Amsterdam (AMC) between July 2010 and August 2012. The study protocol was approved by the Medical Ethics Committee of the AMC and the Competent Authority of the Netherlands. A monitor verified that the trial was performed in accordance with the protocol described in the European Medicine Agency’s “Note for guidance on good clinical practice CPMP/ICH/135/95” as well as the Declaration of Helsinki. Monitoring was performed and reported by following the sponsor’s standing operating procedures.

The nutrition protocol was described previously (16). In brief, patients were randomly assigned to receive enteral nutrition (n = 12), parenteral nutrition (n = 10), or the standard protocol of the Department of Cardio-thoracic Surgery of the AMC that allowed patients to eat until 6 h and drink 2 h before surgery (control group: n = 11). Random assignment was performed online via a secure Internet facility in a 1:1:1 ratio by the TENALEA Clinical Trial Data Management System (version 2.2; National Cancer Institute) by using randomly permuted blocks of sizes 3 and 6. Nutrition was supplemented from 2 d before, during, and until 2 d after surgery. Enteral nutrition consisted of hydrolyzed proteins, carbohydrates, vitamins, and minerals and was given through a peripheral line or peripheral inserted central catheter. Exact compositions of both enteral and parenteral nutrition have been published previously (16). Patients were permitted to eat and drink in addition to their supplemental nutrition.

Primary endpoints of the study were concentrations of amino acids including the arginine:ADMA ratio in myocardial tissue and plasma. The secondary endpoint was myocardial glucose metabolism.

Patients

All patients were undergoing off-pump cardiac surgery for CABG, were aged between 55 and 76 y, and gave written informed consent before study inclusion. Exclusion criteria were a combined valve and CABG procedure, pregnancy, renal insufficiency (defined as a creatinine concentration >95 μmol/L for women and >110 μmol/L for men), and liver insufficiency (defined as alanine aminotransferase concentration >34 U/L for women and >45 U/L for men).

Surgical procedures

Standard anesthetic and off-pump CABG surgical procedures were used in this study.

Myocardial samples

During surgery, 2 tissue samples of the appendix of the right atrium were taken by the surgeon and immediately frozen into liquid nitrogen and stored at −80°C until analysis. One sample was taken after the harvesting of the left mammary artery and before performing the distal anastomosis, and one sample was taken at the end of the procedure before closing of the pericardium.

Blood samples

Blood samples were taken at different time points as follows: 1) before hospital admission after an overnight fast (baseline, before the supplementation of nutrition), 2) before transfer to the operating room; 3) during surgery simultaneously with sampling of the first tissue sample, 4) during surgery simultaneously with sampling of the second tissue sample, 5) the day after surgery, and 6) the third day after surgery after an overnight fast (which was the day after nutrition was ended). After centrifugation, plasma was pipetted and stored at −80°C until additional analysis. To adjust for the effect of hemodilution, plasma concentrations of metabolites in the fourth, fifth, and sixth blood samples were corrected by using the hematocrit according to the following formula:

\[(\text{Metabolite concentration} \times \text{preoperative hematocrit}) ÷ \text{hematocrit at sampling time} \] (1)

Laboratory analysis

In homogenized myocardial tissue (OMNI 2000 homogenizer; OMNI International Inc) and blood plasma, concentrations of
arginine, ADMA, and other amino acids were measured by HPLC by using previously described methods (17–20). Intraassay and interassay CVs for arginine, ADMA, citrulline, and ornithine were <2% and <3%, respectively.

18F-fluorodeoxyglucose positron emission tomography

As described previously (16), myocardial glucose metabolism was noninvasively assessed by using 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography, which was performed 2 wk before and between 4 and 6 wk after CABG. Both regional and total myocardial glucose metabolisms were analyzed by using 18F-FDG standardized uptake values (SUVs). Images were analyzed in a total of 17 myocardial segments and for the left anterior descending coronary artery (LAD), right coronary artery (RCA), and left circumflex coronary artery (RCX) myocardial regions separately (21).

Statistical analysis

Data are expressed as means (±SDs) in case of symmetric data and as medians with IQRs for skewed data. The Kruskal-Wallis test (KW) was used to compare the 3 groups simultaneously to see if there was evidence of differences. If there was a significant difference, it was tested if the difference in the enteral and parenteral groups was larger than in the control group with the MW. The same analyses were done for differences in plasma at the first and fourth blood sampling time points (which were sampled simultaneously with the first tissue sample) were computed. Next, the KW was used to compare the 3 groups simultaneously to see if there was evidence of differences. If there was a significant difference, it was tested if the difference in the enteral and parenteral groups was larger than in the control group with the MW. The same analyses were done for differences in plasma at the first and fourth blood sampling time points (which were sampled simultaneously with the second tissue sample). P < 0.05 (corrected for the number of post hoc tests by using the Holm-Bonferroni method) was considered statistically significant. Correlations were analyzed by using Pearson’s correlation coefficient. Statistical analyses were performed in the statistical package R (version 3.0.1; R Foundation for Statistical Computing).

RESULTS

Patient characteristics

Although 38 patients were enrolled, 33 patients could be analyzed. The CABG procedure of 2 patients switched from off to on pump, the appendix of the right atrium could not be approached in one patient, and the peripheral line was removed in 2 patients. Characteristics of 33 patients are presented in Table 1.

TABLE 1

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Enteral group (n = 12)</th>
<th>Parenteral group (n = 10)</th>
<th>Control group (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preoperative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>66.1 ± 6.12</td>
<td>66.6 ± 6.7</td>
<td>63.3 ± 6.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 (27.0–30.9)</td>
<td>27.1 (25.8–29.7)</td>
<td>27.7 (24.2–30.0)</td>
</tr>
<tr>
<td>Diabetes [n (%)]</td>
<td>3 (25.0)</td>
<td>4 (40.0)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>EuroSCORE²</td>
<td>2.0 (1.3–3.0)</td>
<td>3.0 (2.8–4.0)</td>
<td>2.0 (0.0–3.0)</td>
</tr>
<tr>
<td>Plasma CRP (mg/L)</td>
<td>0.8 (0.7–1.6)</td>
<td>2.7 (1.0–5.6)</td>
<td>0.6 (0.6–3.2)</td>
</tr>
<tr>
<td>Plasma albumin (g/L)</td>
<td>46.5 (45.3–48.8)</td>
<td>43.5 (41.0–48.3)</td>
<td>46.0 (45.0–48.0)</td>
</tr>
<tr>
<td>Plasma NT-proBNP (ng/L)</td>
<td>234 (54–522)</td>
<td>192 (99–391)</td>
<td>99 (71–431)</td>
</tr>
<tr>
<td><strong>Intraoperative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propofol use [n (%)]</td>
<td>8 (66.7)</td>
<td>4 (40)</td>
<td>8 (72.7)</td>
</tr>
<tr>
<td>Surgery duration (min)</td>
<td>268 ± 39</td>
<td>279 ± 26</td>
<td>294 ± 40</td>
</tr>
<tr>
<td><strong>Postoperative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MB (µg/L)</td>
<td>9.0 (6.4–15.0)</td>
<td>8.4 (6.7–9.5)</td>
<td>10.3 (7.6–15.5)</td>
</tr>
<tr>
<td>Intensive care stay (h)</td>
<td>21.5 (18.0–23.8)</td>
<td>23.5 (20.8–29.3)</td>
<td>21.0 (20.0–22.0)</td>
</tr>
<tr>
<td>Stent [n (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Catherization &lt;3 mo [n (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Revascularization &lt;3 mo [n (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Infections &lt;3 mo [n (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Mortality [n (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

1 CK-MB, creatine kinase–myocardial band; CRP, C-reactive protein; EuroSCORE, European System for Cardiac Operation Risk Evaluation score; NT-proBNP, N-terminal pro-Brain Natriuretic Peptide.

²Median ± SD (all such values).

³Median: IQR in parentheses (all such values).

⁴EuroSCORE is a validated risk-stratification system to determine the risk profile for the mortality of cardiothoracic surgery patients (22).
### TABLE 2

Arginine:ADMA ratio and amino acid concentrations in myocardial tissue samples in study groups

<table>
<thead>
<tr>
<th></th>
<th>Start of surgery (first tissue sample)</th>
<th>End of surgery (second tissue sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enteral group (n = 12)</td>
<td>Parenteral group (n = 10)</td>
</tr>
<tr>
<td>Arginine (nmol/g tissue)</td>
<td>87.0 (76.2–104.0)</td>
<td>108.3 (91.8–136.5)</td>
</tr>
<tr>
<td>Arginine:ADMA ratio</td>
<td>92.6 (58.0–106.3)</td>
<td>95.3 (73.8–113.1)</td>
</tr>
<tr>
<td>ADMA (nmol/g tissue)</td>
<td>0.96 (0.77–1.14)</td>
<td>1.23 (0.94–1.36)</td>
</tr>
<tr>
<td>Glutamic acid (nmol/g tissue)</td>
<td>3617 (2724–4338)</td>
<td>3274 (2620–3906)</td>
</tr>
<tr>
<td>Asparagine (nmol/g tissue)</td>
<td>882 (68.0–120.0)</td>
<td>71.8 (45.8–91.8)</td>
</tr>
<tr>
<td>Serine (nmol/g tissue)</td>
<td>238 (186–280)</td>
<td>255 (203–317)</td>
</tr>
<tr>
<td>Glutamine (nmol/g tissue)</td>
<td>1232 (1097–1562)</td>
<td>1076 (842–1526)</td>
</tr>
<tr>
<td>Histidine (nmol/g tissue)</td>
<td>152 (110–168)</td>
<td>130 (107–158)</td>
</tr>
<tr>
<td>Glycine (nmol/g tissue)</td>
<td>397 (257–569)</td>
<td>500 (373–646)</td>
</tr>
<tr>
<td>Threonine (nmol/g tissue)</td>
<td>219 (129–259)</td>
<td>191 (167–231)</td>
</tr>
<tr>
<td>Citrulline (nmol/g tissue)</td>
<td>27.1 (14.9–34.9)</td>
<td>22.9 (16.0–31.5)</td>
</tr>
<tr>
<td>Alanine (nmol/g tissue)</td>
<td>953 (690–1139)</td>
<td>1039 (806–1211)</td>
</tr>
<tr>
<td>Taurine (nmol/g tissue)</td>
<td>6395 (3840–7549)</td>
<td>5982 (4572–6954)</td>
</tr>
<tr>
<td>Tyrosine (nmol/g tissue)</td>
<td>83.8 (57.1–105.6)</td>
<td>72.3 (58.2–83.5)</td>
</tr>
<tr>
<td>Valine (nmol/g tissue)</td>
<td>264 (157–202)</td>
<td>206 (187–272)</td>
</tr>
<tr>
<td>Methionine (nmol/g tissue)</td>
<td>45.9 (25.9–251.6)</td>
<td>57.8 (32.8–207.6)</td>
</tr>
<tr>
<td>Isoleucine (nmol/g tissue)</td>
<td>203 (128–268)</td>
<td>212 (171–263)</td>
</tr>
<tr>
<td>Phenylalanine (nmol/g tissue)</td>
<td>215 (162–383)</td>
<td>298 (235–337)</td>
</tr>
<tr>
<td>Tryptophan (nmol/g tissue)</td>
<td>15.61 (11.41–19.55)</td>
<td>14.32 (12.11–18.01)</td>
</tr>
<tr>
<td>Leucine (nmol/g tissue)</td>
<td>173 (105–203)</td>
<td>132 (112–168)</td>
</tr>
<tr>
<td>Omithine (nmol/g tissue)</td>
<td>39.5 (21.1–42.8)</td>
<td>37.0 (31.4–51.8)</td>
</tr>
<tr>
<td>Lysine (nmol/g tissue)</td>
<td>206 (149–246)</td>
<td>189 (155–226)</td>
</tr>
<tr>
<td>EAAAs (nmol/g tissue)</td>
<td>1487 (1048–1850)</td>
<td>1538 (1204–1858)</td>
</tr>
<tr>
<td>NEAAs (nmol/g tissue)</td>
<td>13,756 (9206–16,029)</td>
<td>12,413 (10,116–15,356)</td>
</tr>
<tr>
<td>BCAAs (nmol/g tissue)</td>
<td>577 (411–747)</td>
<td>563 (464–683)</td>
</tr>
<tr>
<td>Total amino acids (nmol/g tissue)</td>
<td>15,234 (10,379–18,152)</td>
<td>13,831 (11,729–17,167)</td>
</tr>
</tbody>
</table>

1 All values are medians; IQRs in parentheses. Differences in the arginine:ADMA ratio and BCAA concentrations between groups were tested by using Kruskal-Wallis and Mann-Whitney U tests and within groups by using Wilcoxon’s test. *Significant enteral and parenteral groups compared with the control group; †significant first compared with second tissue samples. ADMA, asymmetric dimethylarginine; BCAA, branched chain amino acid; EAA, essential amino acid; NEAA, nonessential amino acid.

2 n = 11.
Myocardial tissue

The mean time between sampling of the first and second tissue sample was 97 ± 28 min. The arginine:ADMA ratio and concentrations of ADMA and other amino acids in the first and second myocardial tissue samples are shown in Table 2.

Differences between groups

The arginine:ADMA ratio in the first tissue sample did not differ significantly between groups (P = 0.14; KW). In the second tissue sample, the arginine:ADMA ratio differed significantly between groups (P = 0.018; KW) and was significantly higher in the enteral and parenteral groups than in the control group (P = 0.012 and P = 0.004, respectively; MW). The BCAA concentrations in the first and second tissue sample did not differ significantly between groups (P = 0.62 and P = 0.58, respectively; KW).

Differences within groups

The arginine:ADMA ratio increased significantly in all groups (enteral: P < 0.001; parenteral: P < 0.001; control: P = 0.009; Wilcoxon’s test). The BCAA concentrations increased significantly from the first to second tissue samples in all groups (enteral: P = 0.021; parenteral: P = 0.010; control group: P = 0.026; Wilcoxon’s test).

Blood plasma

Boxplots with the arginine:ADMA ratio in plasma in the enteral, parenteral, and control groups are shown in Figure 1. Likewise, the BCAA concentration in plasma is shown in Figure 2. For both the arginine:ADMA ratio and BCAA concentration, the median graph line showed a similar pattern of an increase to time point 4 (ie, the end of surgery) and a drop to time point 5 (ie, the day after surgery). Except from the last time point, graph lines of enteral and parenteral groups were above graphs lines of the control group.

To show changes in the plasma arginine:ADMA ratio from baseline until the start of surgery (ie, time point 3) and from baseline until the end of surgery (ie, time point 4), changes in third minus first blood samples and fourth minus first blood samples, respectively, in each study group are shown in Figure 3. When we analyzed the change in the first and third blood samples, no significant differences were shown between groups (P = 0.29; KW). The change in the first and fourth blood samples differed significantly between groups (P = 0.008; KW) and was significantly higher in the enteral and parenteral groups than in the control group (P = 0.001 and P = 0.013, respectively; MW). Likewise, these changes in plasma BCAA concentrations are shown in Figure 4. Both change in the first and third blood samples and first and fourth blood samples differed significantly between groups (both P < 0.001; KW) and were significantly higher in the enteral and parenteral groups than in the control.
Myocardial glucose metabolism

No significant differences in $^{18}$F-FDG uptake were shown between the 3 groups. Therefore, for an additional analysis, all patients were grouped together. The maximal myocardial $^{18}$F-FDG uptake was $9.94 \pm 3.73$ SUVs before surgery and $9.70 \pm 3.89$ SUVs after surgery. For the LAD, RCX, and RCA regions, SUVs were $7.8 \pm 3.1$, $8.1 \pm 3.1$, and $7.4 \pm 3.0$, respectively, before surgery, and $7.9 \pm 3.4$, $7.6 \pm 3.1$, and $7.3 \pm 3.2$, respectively, after surgery. For example, $^{18}$F-FDG positron emission tomography images of a patient in the enteral group are shown in Figure 5. The patient had a preoperative SUVmax of 12.8 that increased to an SUVmax of 16.1 after surgery. $^{18}$F-FDG uptake increased from 8.4 (SUVmax = 9.7) to 14.0 (SUVmax = 15.8) SUVs in the LAD region, 11.3 (SUVmax = 12.8) to 13.8 (SUVmax = 16.1) SUVs in the RCX region, and 8.0 (SUVmax = 9.5) to 10.3 (SUVmax = 12.8) SUVs in the RCA region.

The change in plasma arginine:ADMA from baseline (firsts blood sample) to the day after nutrition was ended (sixth blood sample, 3 d after surgery) was positively correlated with the change in preoperative to postoperative in maximal $^{18}$F-FDG uptake ($r = 0.443$, $P = 0.027$) and $^{18}$F-FDG uptake for each region (LAD: $r = 0.440$, $P = 0.028$; RCX: $r = 0.429$, $P = 0.032$; RCA: $r = 0.379$, $P = 0.062$) (Figure 6).

DISCUSSION

To our knowledge, this is the first study in which the effect of supplementation of enteral and parenteral nutrition during surgery on the human heart was investigated. Our results showed that nutrition before and during surgery augmented the myocardial and plasma arginine:ADMA ratio and plasma BCAA concentrations. Furthermore, the change in plasma arginine:ADMA from preoperative to postoperative was positively correlated with the change in myocardial glucose metabolism.

The arginine:ADMA ratio has been proposed to be a better indicator of NO availability than either arginine or ADMA separately because the ratio reflects the proportion of NOS substrate and inhibitor (4). Accordingly, a low arginine:ADMA ratio predicted mortality in patients with cardiac failure (6, 23). A reduction of the ratio decreased cardiac output and diminished the flow in the microcirculation of major organs in animals (24). However, one study did not show an association between the arginine:ADMA ratio and risk of secondary cardiovascular disease events in patients with stable coronary heart disease,
which suggested that there might be other factors involved that interfere with NO availability (25). In contrast, the arginine:ADMA ratio might explain results of studies that did not show an association between ADMA and cardiovascular function. For example, ADMA was not associated with impaired right ventricular function in patients with systemic sclerosis (26). The study by Ciurzyński et al (26) measured the inhibitor of NOS (ie, the plasma ADMA concentration) but did not describe the plasma arginine concentration, which would have given information about the substrate of NOS. In the study by Ciurzyński et al (26), a relatively high arginine concentration may have overcome detrimental effects of ADMA by

FIGURE 3. Boxplots of the δ arginine/ADMA ratio in plasma of third minus first blood samples (upper graph) and fourth minus first blood samples (bottom graph) of each study group. Upper graph: no significant differences were shown between groups (P = 0.29; Kruskal-Wallis test). Bottom graph: δ differed significantly between groups (P = 0.008; Kruskal-Wallis test) and was significantly higher in the enteral and parenteral groups than in the control group (P = 0.001 and P = 0.013, respectively; Mann-Whitney U test). ADMA, asymmetric dimethylarginine; *, outlier.

FIGURE 4. Boxplots of the δ BCAA concentration in plasma of third minus first blood samples (upper graph) and fourth minus first blood samples (bottom graph) of each study group. Upper graph: δ differed significantly between groups (P < 0.001; Kruskall-Wallis test) and was significantly higher in the enteral and parenteral groups than in the control group (both P < 0.001; Mann-Whitney U test). Bottom graph: δ differed significantly between groups (P < 0.001; Kruskal-Wallis test) and was significantly higher in the enteral and parenteral groups than in the control group (both P < 0.001; Mann-Whitney U test). BCAA, branched chain amino acid; *, outlier.
reversing the competitive inhibition of NOS by ADMA. A large number of studies investigated the effect of either increasing arginine (27) or decreasing ADMA (4) concentrations. An increase of plasma arginine can improve cardiovascular function, whereas the effects on the clinical outcome are not yet clear (4, 27). Studies that decreased plasma ADMA have shown beneficial effects for cardiovascular function (4), whereas the long-term effects are still undescribed. In our patients, revascularization was performed to improve myocardial perfusion. However, an increase in myocardial perfusion is only valuable if it result in an improvement of cardiac function. In our study, an increase in the preoperative to postoperative plasma arginine:ADMA ratio was positively correlated with an increase in myocardial glucose metabolism. These findings suggest that revascularization in combination with an increase in the arginine:ADMA ratio enhances myocardial viability (ie, glucose metabolism). Probably the extra flow induced by NO elevation in addition to revascularization further improves access of substrates to cardiac cells reflected by an increase in glucose uptake. Because our enteral and parenteral nutrition increased both myocardial and plasma arginine:ADMA, our nutritional intervention might indirectly have enhanced myocardial glucose metabolism.

When we focused on amino acids, the concentration of BCAAs in myocardial tissue increased during surgery in all study groups. In addition, higher changes were seen in plasma BCAA concentrations during surgery in the enteral and parenteral groups than in the control group. These results suggest that both enteral and parenteral nutrition are able to increase plasma concentrations of BCAAs. Unfortunately, our findings did not give a decisive answer about whether increases of myocardial BCAAs are affected by enteral and parenteral nutrition. BCAAs are essential for cardiac protein synthesis and metabolism (9). Previous studies have shown that nutrition is able to influence the myocardial tissue content, whereby protein synthesis in the heart was stimulated by amino acid perfusate (10) and BCAA infusion (11). However, to our knowledge, the effect of nutrition before, during, and after surgery on myocardial tissue was not previously investigated.

Currently, it is common practice that patients receive only clear fluids during the period before surgery and the day after surgery, which would lead to starvation of the patient over a longer period of time. Fasting can induce thirst, stress, insulin resistance (28), and nutrient deficiencies that can impair immune defense (29). In addition, glycogen reserves last only a few hours, implying that fasting gluconeogenesis mainly depends on the amino acid supply by body protein catabolism, which further weakens the patient. Because surgical patients are already in a catabolic state (30), prolonged fasting will impair recovery after surgery (28). Therefore, it can be hypothesized that avoiding fasting and starvation may be favorable to the surgical patient. Our study, which showed that nutrition before and during surgery increased the myocardial arginine:ADMA ratio, supports this notion.

This study had limitations. First, this study was a proof-of-concept trial that tested the novel strategy of nutrition during surgery as a perioperative treatment. The sample size was powered for the primary outcome (ie, the arginine concentration in myocardial tissue) and a significant difference between enteral, parenteral, and control groups. Unfortunately, the intended sample size could not be reached because of low consent numbers. Nevertheless, the study showed that it is feasible to supply nutrition during surgery, which can increase the arginine:ADMA ratio in myocardial tissue and plasma. Second, baseline values of myocardial tissue concentrations could not be measured in the nutritional groups because it was not possible to take myocardial biopsies before surgery. Furthermore, it is unknown whether ADMA and other amino acids are representatively and equally distributed in the right atrial appendix compared with in other parts of the myocardium. Finally, in this study, it was assumed that supplementing arginine (and its precursor glutamine) as components of the formulated nutrition increased arginine concentrations in the heart. However, this result could be
assured only by the use of isotope-labeled arginine. Therefore, results of our study should be interpreted with caution until other studies have reproduced and cross-validated these findings.

In conclusion, this study shows that enteral and parenteral nutrition before, during, and after surgery increases the arginine:ADMA ratio in the human heart and plasma and increases plasma BCAA concentrations. Furthermore, an increase in plasma arginine:ADMA was associated with an increase in myocardial glucose metabolism. No adverse outcome was observed when nutrition was supplied during surgery. These findings justify studies to investigate whether nutritional supplementation during surgery has an independent or additional effect on cardiac recovery and the clinical outcome.

We thank the following individuals: attending nurses, physicians, residents, and nonmedical staff of the Departments of Cardio-thoracic Surgery, Anaesthesiology, Intensive Care Unit (ICU), Nuclear Medicine, and Pharmacy at the AMC for their help with patient care and protocol compliance; Max Ramali from the Department of Gastroenterology at the AMC for the placement and verification of the duodenal tubes; Jan van Straalen from the Department of Chemistry at the AMC for handling of the blood and tissue samples; Sigrid de Jong from the Department of Clinical Chemistry of the VU University Medical Center for expert assistance in the analysis of tissue and plasma amino acids; and Sarah Kidwai and Dominique Wanders, who were students at the time of the project, for their assistance in data collection. Last, we thank all participants who were willing to participate in the study.

The authors’ responsibilities were as follows—MV: was responsible for protocol, patient selection, random assignment, data collection, and writing of the manuscript; MD: participated in data collection and tissue analysis and reviewed the manuscript; HJV: participated in the development of the protocol and positron emission tomography analysis and reviewed the manuscript; WEMK: participated in the development of the protocol and selection of patients and reviewed the manuscript; RC: participated in the selection of the patients, performed operations, and reviewed the manuscript; RT: participated in protocol compliance at the ICU and reviewed the manuscript; EMK: coordinated parenteral nutrition preparation and reviewed the manuscript; TT: participated in the tissue analysis and reviewed the manuscript; MAJ: performed the statistical analysis; WW: reviewed the manuscript; and BAJMdM and PAMvL: participated in the development of the protocol, reviewed the manuscript, and had responsibility for the final content of the manuscript. None of the authors had a conflict of interest.

FIGURE 6. Correlations between the change in plasma arginine/ADMA ratio and glucose metabolism in the total myocardium (upper left graph), LAD (upper right graph), RCA (bottom left graph), and RCX (bottom right graph) from preoperative to postoperative. ADMA, asymmetric dimethylarginine; LAD, left anterior descending coronary artery; RCA, right coronary artery; RCX, left circumflex coronary artery; 18F-FDG, 18F-fluorodeoxyglucose.
REFERENCES

1. Umar S, van der Laarse A. Nitric oxide and nitric oxide synthase
   isoforms in the normal, hypertrophic, and failing heart. Mol Cell

2. Tsuei BJ, Bernard AC, Shane MD, Shirley LA, Maley ME, Boulanger
   BR, Kearney PA, Ochoa JB. Surgery induces human mononuclear cell

3. Kaye DM, Ahlers BA, Autelitano DJ, Chin-Dusting JP. In vivo and
   in vitro evidence for impaired arginine transport in human heart failure.
   Circulation 2000;102:2707–12.

4. Visser M, Paulus WJ, Vermeulen MA, Richir MC, Davids M, Wisse-
   link W, de Mol BA, van Leeuwen PA. The role of asymmetric dime-
   thylarginine and arginine in the failing heart and its vasculature. Eur J

5. Nijveldt RJ, Teerlink T, van der Hoven B, Siroen MP, Kuik DJ, Rau-
   werda JA, van Leeuwen PA. Asymmetrical dimethylarginine (ADMA)
   in critically ill patients: high plasma ADMA concentration is an

6. Visser M, Vermeulen MA, Richir MC, Teerlink T, Houdijk AP, Kost-
   tense PJ, Wisselink W, de Mol BA, van Leeuwen PA, Oudemans-van
   Straaten HM. Imbalance of arginine and asymmetric dimethylarginine
   is associated with markers of circulatory failure, organ failure and

7. Seljeflot I, Nilsson BB, Westheim AS, Bratseth V, Arnesen H. The L-
   arginine-asymmetric dimethylarginine ratio is strongly related to the
   Fail 2011;17:135–42.


9. Taegtmeyer H, Harinstein ME, Gheorghiade M. More than bricks and
   mortar: comments on protein and amino acid metabolism in the heart.
   Am J Cardiol 2008;101:3E–7E.

    Surgery induces human mononuclear cell arginase I expression.

11. Young LH, McNulty PH, Morgan C, Deckelbaum LI, Zaret BL, Barrett
    EJ. Myocardial protein turnover in patients with coronary artery dis-
    87:554–60.

    Leeuwen PA. Low arginine plasma levels on protein synthesis. J Biol

13. Tsuei BJ, Bernard AC, Shane MD, Shirley LA, Maley ME, Boulanger
    BR, Kearney PA, Ochoa JB. Surgery induces human mononuclear cell

14. Young LH, McNulty PH, Morgan C, Deckelbaum LI, Zaret BL, Barrett
    EJ. Myocardial protein turnover in patients with coronary artery dis-
    87:554–60.

15. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey
    WK, Pennell DJ, Rumberger JA, Ryan T, Verani MS. Standardized
    myocardial segmentation and nomenclature for tomographic imaging
    of the heart. A statement for healthcare professionals from the Cardiac
    Imaging Committee of the Council on Clinical Cardiology of the

    PG, Blom HJ, Teerlink T. Simultaneous determination of asymmetric
    and symmetric dimethylarginine, L-monomethylarginine, L-arginine,
    and L-homoarginine in biological samples using stable isotope dilution
    liquid chromatography tandem mass spectrometry. J Chromatogr B

17. de Jong S, Teerlink T. Analysis of asymmetric dimethylarginine in
    plasma by HPLC using a monoionic column. Anal Biochem 2006;353:
    287–9.

18. Neubauer S, van der Laarse A. Nitric oxide and nitric oxide synthase
   isoforms in the normal, hypertrophic, and failing heart. Mol Cell

19. Visser M, Paulus WJ, Vermeulen MA, Richir MC, Davids M, Wisse-
   link W, de Mol BA, van Leeuwen PA. The role of asymmetric dime-
   thylarginine and arginine in the failing heart and its vasculature. Eur J

20. Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PA. Determination of
    arginine, asymmetric dimethylarginine, and symmetric dimethylarginine
    in human plasma and other biological samples by high-performance

21. Anderssohn M, Rosenberg M, Schwedhelm E, Zugck C, Lutz M,
    Laneburg N, Frey N, Bojer RH. The L-Arginine-asymmetric dimethyl-
    arginine ratio is an independent predictor of mortality in dilated

22. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey
    WK, Pennell DJ, Rumberger JA, Ryan T, Verani MS. Standardized
    myocardial segmentation and nomenclature for tomographic imaging
    of the heart. A statement for healthcare professionals from the Cardiac
    Imaging Committee of the Council on Clinical Cardiology of the

23. Anderssohn M, Rosenberg M, Schwedhelm E, Zugck C, Lutz M,
    Laneburg N, Frey N, Bojer RH. The L-Arginine-asymmetric dimethyl-
    arginine ratio is an independent predictor of mortality in dilated

24. Visser M, Paulus WJ, Vermeulen MA, Richir MC, Davids M, Wisse-
    link W, de Mol BA, van Leeuwen PA. The role of asymmetric dime-
    thylarginine and arginine in the failing heart and its vasculature. Eur J

25. Visser M, Paulus WJ, Vermeulen MA, Richir MC, Davids M, Wisse-
    link W, de Mol BA, van Leeuwen PA. The role of asymmetric dime-
    thylarginine and arginine in the failing heart and its vasculature. Eur J

26. Visser M, Paulus WJ, Vermeulen MA, Richir MC, Davids M, Wisse-
    link W, de Mol BA, van Leeuwen PA. The role of asymmetric dime-
    thylarginine and arginine in the failing heart and its vasculature. Eur J

27. Nijveldt RJ, Prins HA, Siroen MP, Rauwerda JA, Teerlink T, van
    Leeuwen PA. Low arginine plasma levels on protein synthesis. J Biol

28. Visser M, Paulus WJ, Vermeulen MA, Richir MC, Davids M, Wisse-
   link W, de Mol BA, van Leeuwen PA. The role of asymmetric dime-
   thylarginine and arginine in the failing heart and its vasculature. Eur J

29. Visser M, Paulus WJ, Vermeulen MA, Richir MC, Davids M, Wisse-
   link W, de Mol BA, van Leeuwen PA. The role of asymmetric dime-
   thylarginine and arginine in the failing heart and its vasculature. Eur J