Nutrition before, during, and after surgery increases the arginine:asymmetric dimethylarginine ratio and relates to improved myocardial glucose metabolism: a randomized controlled trial[1–3]

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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. ^{18}F-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 enteral and parenteral groups than 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 *Am J Clin Nutr* 2014;99:1440–9.

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

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[4] Abbreviations 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; ^{18}F-FDG, ^{18}F-fluorodeoxyglucose.

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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 is given before, during, and after surgery have not been previously been performed. Cardiac surgery patients undergoing off-pump (ie, without a cardiopulmonary bypass) coronary artery bypass grafting (CABG) offer the possibility to study myocardial tissue samples that are not influenced by cardioplegic effects. In this study, we investigated whether 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 computerized guidance system–placed nasoduodenal tube. Parenteral nutrition (aseptically prepared) consisted of amino acids, lipids, glucose, 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:

$$(\text{Metabolite concentration} \times \text{preoperative hematocrit}) \div \text{hematocrit at sampling time} \quad (1)$$

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.

^{18}F-fluorodeoxyglucose positron emission tomography

As described previously (16), myocardial glucose metabolism was noninvasively assessed by using ^{18}F-fluorodeoxyglucose (^{18}F-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 ^{18}F-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 between the arginine:ADMA ratio in the first tissue. If there was a significant difference, the Mann-Whitney *U* test (MW) was used to test the alternative hypothesis that the ratio in the enteral group was higher than in the control group. The same procedure was done for the parental group. The procedure was repeated for the second tissue. For all groups separately, it was tested whether the arginine:ADMA ratio was higher in the second tissue than the first tissue. The Wilcoxon's signed rank test was used. The same analyses were done for the BCAA concentration in myocardial tissue samples. Furthermore, for every group (the enteral, parenteral, and control groups), the difference of the arginine:ADMA ratio and BCAA concentrations in plasma at first and third 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 were 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**. As evaluated by clinical judgment, at baseline, no clinically important differences were observed between groups.

TABLE 1
Patient characteristics[1]

	Enteral group (n = 12)	Parenteral group (n = 10)	Control group (n = 11)
Preoperative			
Age (y)	66.1 ± 6.1[2]	66.6 ± 6.7	63.3 ± 6.7
Sex (M) [n (%)]	12 (100)	10 (100)	11 (100)
BMI (kg/m^2)	27.8 (27.0–30.9)[3]	27.1 (25.8–29.7)	27.7 (24.2–30.0)
Diabetes [n (%)]	3 (25.0)	4 (40.0)	3 (27.3)
EuroSCORE[4]	2.0 (1.3–3.0)	3.0 (2.8–4.0)	2.0 (0.0–3.0)
Plasma CRP (mg/L)	0.8 (0.7–1.6)	2.7 (1.0–5.6)	0.6 (0.6–3.2)
Plasma albumin (g/L)	46.5 (45.3–48.8)	43.5 (41.0–48.3)	46.0 (45.0–48.0)
Plasma NT-proBNP (ng/L)	234 (54–522)	192 (99–391)	99 (71–431)
Intraoperative			
Propofol use [n (%)]	8 (66.7)	4 (40)	8 (72.7)
Surgery duration (min)	268 ± 39	279 ± 26	294 ± 40
Postoperative			
CK-MB (μg/L)	9.0 (6.4–15.0)	8.4 (6.7–9.5)	10.3 (7.6–15.5)
Intensive care stay (h)	21.5 (18.0–23.8)	23.5 (20.8–29.3)	21.0 (20.0–22.0)
Stent [n (%)]	0 (0)	0 (0)	0 (0)
Catheterization <3 mo [n (%)]	0 (0)	0 (0)	1 (9.1)
Revascularization <3 mo [n (%)]	0 (0)	0 (0)	0 (0)
Infections <3 mo [n (%)]	0 (0)	0 (0)	1 (9.1)
Mortality [n (%)]	0 (0)	0 (0)	0 (0)

[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.
[2] Mean ± SD (all such values).
[3] Median; IQR in parentheses (all such values).
[4] EuroSCORE 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[1]

	Start of surgery (first tissue sample)			End of surgery (second tissue sample)		
	Enteral group (n = 12)	Parenteral group (n = 10)	Control group (n = 11)	Enteral group (n = 12)	Parenteral group (n = 10)	Control group (n = 11)
Arginine:ADMA ratio	92.6 (58.0–106.3)[2]	95.3 (73.8–113.1)	67.0 (54.3–95.9)	115.0 (98.0–142.2)*,†	116.9 (100.3–135.3)*,†	93.3 (82.7–101.1)†
Arginine (nmol/g tissue)	87.0 (76.2–104.0)	108.3 (91.8–136.5)	84.5 (73.5–91.5)	124.8 (85.0–155.7)	137.9 (121.4–160.8)	104.5 (92.0–139.2)
ADMA (nmol/g tissue)	0.96 (0.77–1.14)[2]	1.23 (0.94–1.36)	1.03 (0.81–1.25)	1.05 (0.83–1.25)	1.28 (1.02–1.59)	1.22 (0.97–1.34)
Glutamic acid (nmol/g tissue)	3617 (2724–4338)	3274 (2620–3906)	3139 (2325–3654)	1861 (1672–2184)	1940 (1470–2635)	2981 (1939–3219)
Asparagine (nmol/g tissue)	88.2 (68.0–120.0)	71.8 (45.8–91.8)	75.1 (58.8–104.1)	98.3 (78.4–114.2)	86.0 (57.9–102.0)	87.4 (69.8–96.9)
Serine (nmol/g tissue)	238 (186–280)	255(203–317)	271 (214–343)	278 (236–341)	349 (249–455)	312 (250–485)
Glutamine (nmol/g tissue)	1232 (1097–1562)	1076 (842–1526)	1231 (933–1495)	1092 (1020–1361)	902 (805–1562)	1272 (925–1394)
Histidine (nmol/g tissue)	152 (110–168)	130 (107–158)	137 (103–188)	139 (109–173)	148 (117–207)	151 (134–159)
Glycine (nmol/g tissue)	397 (257–569)	500 (373–646)	347 (308–442)	492 (337–561)	646 (413–734)	439 (399–608)
Threonine (nmol/g tissue)	219 (129–259)	191 (167–231)	177 (147–231)	234 (176–266)	230 (190–334)	216 (167–238)
Citrulline (nmol/g tissue)	27.1 (14.9–34.9)	22.9 (16.0–31.5)	19.6 (17.2–21.9)	29.1 (22.4–38.9)	30.2 (17.9–39.1)	25.1 (23.9–31.5)
Alanine (nmol/g tissue)	953 (690–1139)	1039 (806–1211)	1035 (843–1076)	1545 (1002–2432)	1809 (1364–2177)	1244 (897–1915)
Taurine (nmol/g tissue)	6395 (3840–7549)	5982 (4572–6954)	7334 (3914–8324)	4566 (3747–7430)	5172 (3679–7385)	6686 (5087–8045)
Tyrosine (nmol/g tissue)	83.6 (57.1–105.6)	72.3 (58.2–83.8)	72.5 (46.3–80.5)	102.5 (63.5–112.2)	83.6 (63.4–113.1)	81.9 (68.8–89.7)
Valine (nmol/g tissue)	264 (157–302)	206 (187–272)	178 (146–221)	302 (207–336)	256 (225–312)	228 (213–248)
Methionine (nmol/g tissue)	45.9 (25.9–251.6)	57.8 (32.8–207.6)	158.9 (29.9–184.5)	43.0 (35.2–175.7)	64.1 (41.4–387.3)	165.5 (34.5–640.1)
Isoleucine (nmol/g tissue)	203 (128–268)	212 (171–263)	181 (165–252)	267 (157–312)	265 (237–349)	294 (218–388)
Phenylalanine (nmol/g tissue)	215 (162–383)	298 (235–337)	265 (191–336)	289 (197–373)	373 (297–438)	412 (327–589)
Tryptophan (nmol/g tissue)	15.61 (11.41–19.55)	14.32 (12.11–18.01)	8.86 (6.06–22.67)	17.56 (13.57–24.47)	21.21 (13.94–28.13)	15.19 (9.93–23.27)
Leucine (nmol/g tissue)	173 (105–203)	132 (112–168)	124 (107–151)	229 (131–250)	165 (145–243)	171 (145–182)†
Ornithine (nmol/g tissue)	39.5 (21.1–48.6)	37.0 (31.4–51.8)	33.0 (29.6–44.2)	49.3 (35.8–57.1)	46.4 (34.1–97.5)	41.3 (36.7–65.3)
Lysine (nmol/g tissue)	206 (149–246)	189 (155–226)	183 (124–207)	242 (193–276)	251 (199–285)	216 (171–294)
EAAs (nmol/g tissue)	1487 (1048–1850)	1538 (1204–1858)	1426 (1067–1536)	1751 (1531–2040)	2004 (1711–2186)	1884 (1555–2575)
NEAAs (nmol/g tissue)	13,756 (9206– 16,029)	12,413 (10,116–15,356)	13,585 (9886– 18,605)	10,528 (9030– 14,707)	11,857 (9055– 15,337)	12,977 (11,134–14,640)
BCAAs (nmol/g tissue)	577 (411–747)	563 (464–683)	507 (390–616)	771 (604–870)†	715 (597–878)†	695 (528–798)†
Total amino acids (nmol/g tissue)	15,234 (10,379–18,152)	13,831 (11,729–17,167)	15,012 (11,213–20,109)	12,379 (10,540–17,756)	13,873 (10,987–17,344)	14,617 (12,910–16,607)

[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.

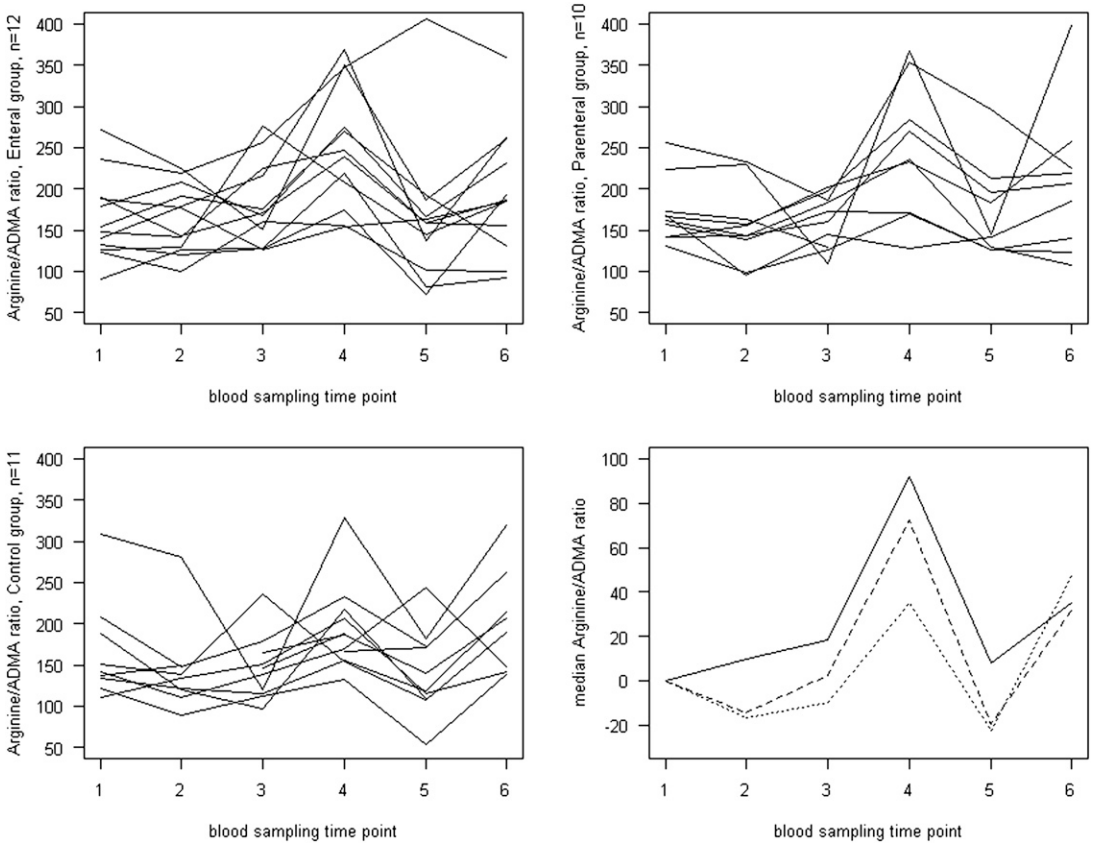

FIGURE 1. The arginine/ADMA ratio in plasma of each patient in the enteral (upper left graph), parenteral (upper right graph), and control (bottom left graph) groups and the median of each group (bottom right graph) during the study period (corrected with baseline concentrations). Blood sampling time points: 1 = baseline, 2 = before surgery, 3 and 4 = during surgery, 5 = first day after surgery, and 6 = third day after surgery. Bottom right graph: ─, enteral group; - - -, parenteral group; ····, control group. ADMA, asymmetric dimethylarginine.

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

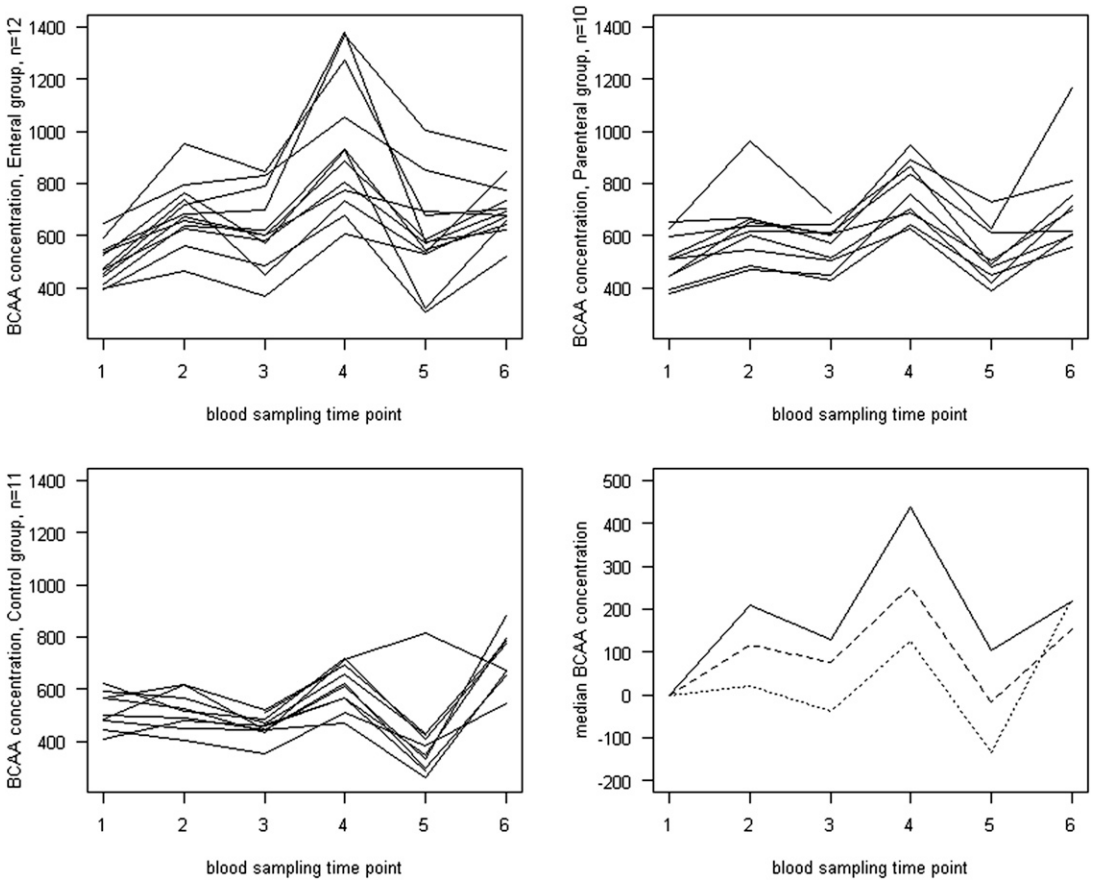

FIGURE 2. BCAA concentrations in plasma of each patient in the enteral (upper left graph), parenteral (upper right graph), and control (bottom left graph) groups and the median of each group (bottom right graph) during the study period (corrected with baseline concentrations). Blood sampling time points: 1 = baseline, 2 = before surgery, 3 and 4 = during surgery, 5 = first day after surgery, and 6 = third day after surgery. Bottom right graph: ─, enteral group; - - -, parenteral group; ····, control group. BCAA, branched-chain amino acid.

group [change in first and third blood samples, both $P < 0.001$ (MW); change in first and fourth blood samples, both $P < 0.001$ (MW)].

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 ± 3.73 SUVs before surgery and 9.70 ± 3.89 SUVs after surgery. For the LAD, RCX, and RCA regions, SUVs were 7.8 ± 3.1, 8.1 ± 3.1, and 7.4 ± 3.0, respectively, before surgery, and 7.9 ± 3.4, 7.6 ± 3.1, and 7.3 ± 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,

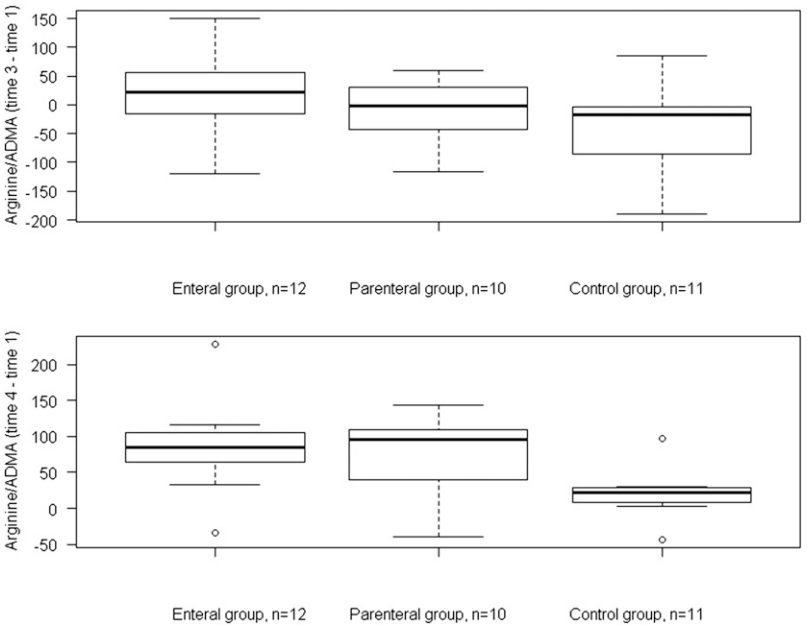

FIGURE 3. Boxplots of the δ arginine/ADMA ratio in plasma of third minus first blood samples (upper graph) and fourth minus first blood samples (bottom graph) of each study group. Upper graph: no significant differences were shown between groups ($P = 0.29$; Kruskal-Wallis test). Bottom graph: δ differed 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.

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

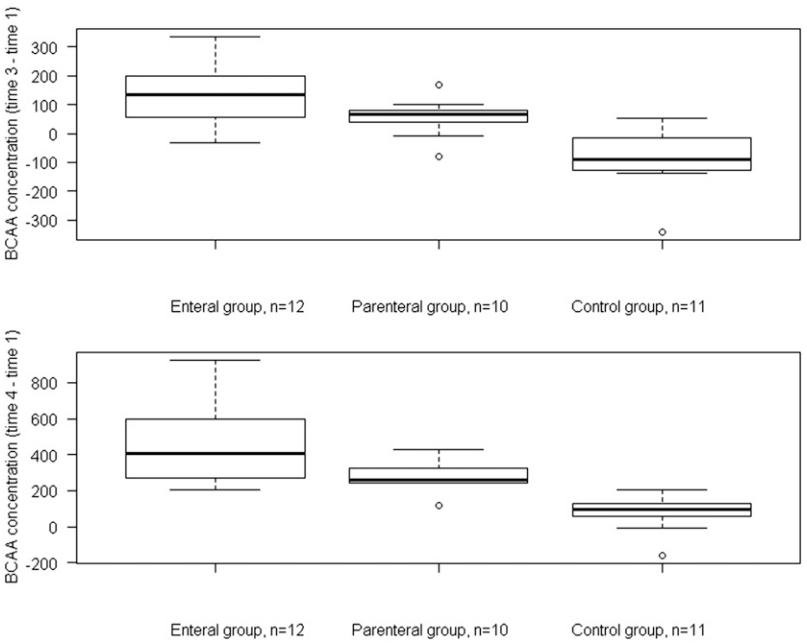

FIGURE 4. Boxplots of the δ BCAA concentration in plasma of third minus first blood samples (upper graph) and fourth minus first blood samples (bottom graph) of each study group. Upper graph: δ differed significantly between groups ($P < 0.001$; Kruskall-Wallis test) and was significantly higher in the 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.

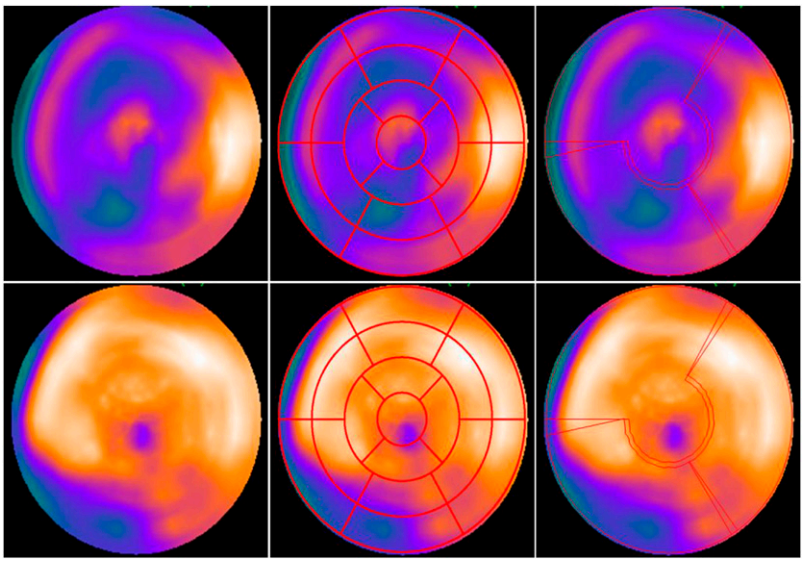

FIGURE 5. Example of preoperative (upper row) and postoperative (lower row) displays of ^{18}F-fluorodeoxyglucose uptake of a study patient in the enteral group in the overall myocardium (first column) according to the 17 myocardial segments model (second column) and the distribution of the 3 major coronary artery perfusion areas (third column). The concentration of ^{18}F-fluorodeoxyglucose myocardial uptake is encoded from low (green-blue) via medium (yellow) to high (white). There was a clear improvement of ^{18}F-fluorodeoxyglucose myocardial uptake after surgery.

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

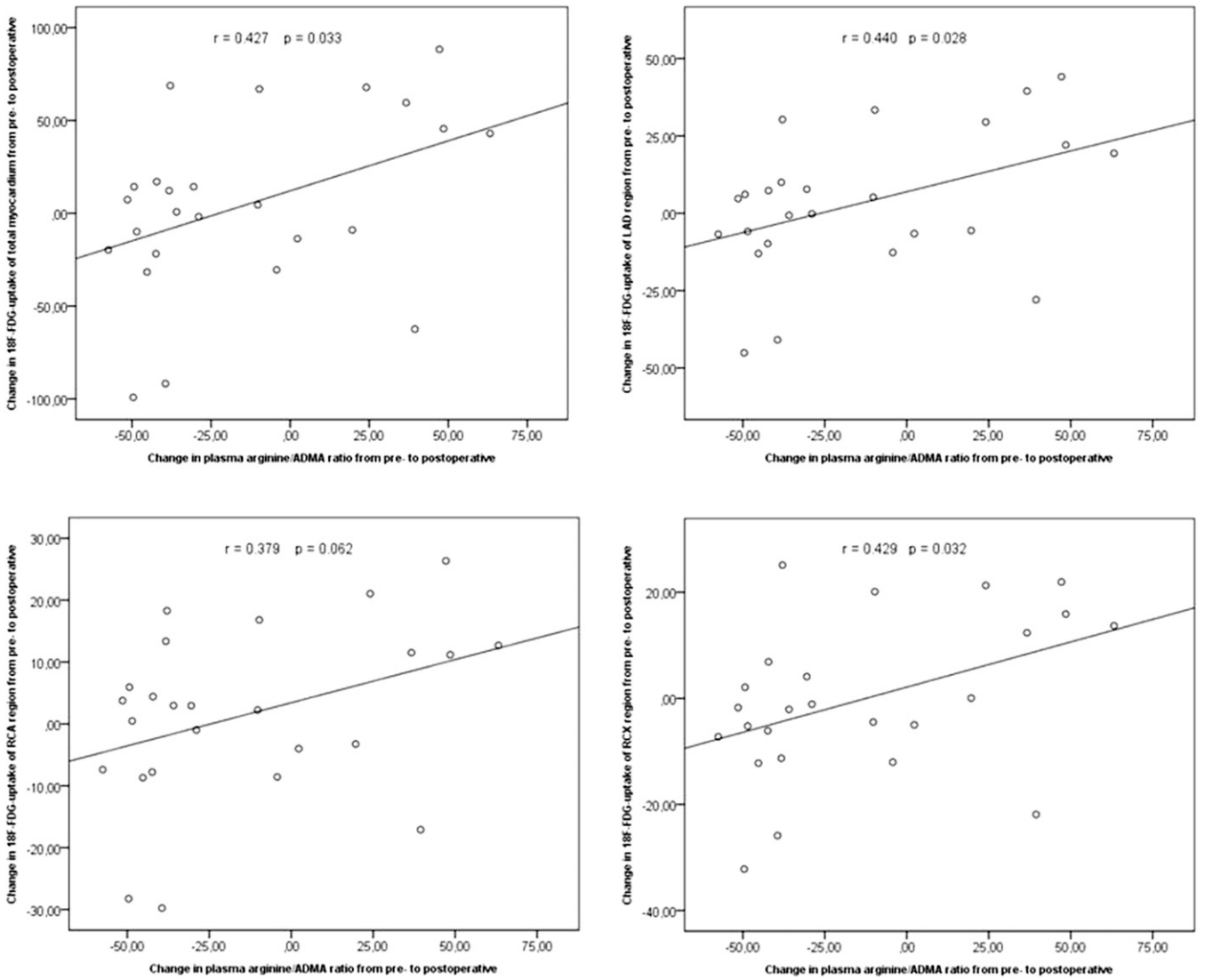

FIGURE 6. Correlations between the change in plasma arginine/ADMA ratio and glucose metabolism in the total myocardium (upper left graph), LAD (upper right graph), RCA (bottom left graph), and RCX (bottom right graph) from preoperative to postoperative. ADMA, asymmetric dimethylarginine; LAD, left anterior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

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.

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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.

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