Acute Right Ventricular Restrictive Physiology After Repair of Tetralogy of Fallot : Association With Myocardial Injury and Oxidative Stress

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Acute Right Ventricular Restrictive Physiology After Repair of Tetralogy of Fallot

Association With Myocardial Injury and Oxidative Stress

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Background—Acute right ventricular (RV) restrictive physiology after tetralogy of Fallot repair results in low cardiac output and a prolonged stay in the intensive care unit (ICU). However, its mechanism remains uncertain.

Methods and Results—In the first 24 hours after tetralogy of Fallot repair (n=11 patients), serial prospective measurements were performed of cardiac troponin T, indexes of NO production (NO_2^- and NO_3^- combined as NOx), and iron metabolism and antioxidants. RV diastolic function was assessed by transthoracic Doppler echocardiography. Patients who had a long stay in the ICU were characterized by restrictive RV physiology (nonrestrictive group [n=7]: 3.0±0.6 days [mean±SD]; restrictive group [n=4]: 10.7±3.1 days). Troponin T peak concentration and the area under its concentration-time curve (AUC) were higher in the restrictive RV group (peak: restrictive group 17.0±2.8 μg/L, nonrestrictive group 10.4±4.6 μg/L, $P<0.03$; AUC: restrictive group 268.8±73.6 $\mu g \cdot h^{-1} \cdot L^{-1}$, nonrestrictive group 136.2±48.3 $\mu g \cdot h^{-1} \cdot L^{-1}$, $P<0.03$). Plasma NOx/creatinine concentrations were higher in the restrictive group than the nonrestrictive group at 2 hours after bypass (restrictive group 1.3±0.4, nonrestrictive group 0.8±0.2; $P=0.04$) but were similar by 24 hours. Iron loading peaked 2 to 10 hours after bypass and was more severe in the restrictive group (peak transferrin saturation: restrictive group 83.9±13.0%, nonrestrictive group 58.3±16.2%, $P=0.05$; minimum total iron-binding capacity: restrictive group 0.59±0.21%, nonrestrictive group 0.76±0.06%, $P=0.04$; minimum iron-binding antioxidant activity to oxyorganic radicals: restrictive group 9.5±22.4%, nonrestrictive group 50.6±11.4%, $P=0.01$).

Conclusions—After tetralogy of Fallot repair, acute restrictive RV physiology is associated with greater intraoperative myocardial injury and postoperative oxidative stress with severe iron loading of transferrin. **(*Circulation*. 1999;100:1540-1547.)**

Key Words: tetralogy of Fallot ■ ventricles ■ diastole ■ free radicals

After tetralogy of Fallot repair in late infancy or early childhood, the majority of children have an uneventful postoperative course, with an intensive care unit (ICU) stay of 2 to 3 days and early mortality of <1% in the best series.[1] A subgroup of patients have a distinctly different course characterized by low cardiac output necessitating prolonged ventilation, inotropic support, and intensive care for 7 to 10 days. These patients were previously demonstrated to have acute right ventricular (RV) restrictive physiology, characterized by antegrade late diastolic flow in the pulmonary artery; ie, atrial contraction is transmitted to the pulmonary artery, and the stiff RV acts as a passive conduit with little or no true RV filling during this period of diastole.[2] This is a transient phenomenon that is resolved within 2 weeks, coincident with clinical improvement.[2,3] The origin of acute RV restriction is unknown. Intraoperatively, the tetralogy of Fallot RV may be inadequately protected,[4,5] because its anterior position makes satisfactory hypothermia difficult,[6] and hypertrophy complicates the homogeneous delivery of cardioplegia. Despite these considerations, previous studies[2,3] have failed to demonstrate a relationship between acute RV restriction and simple measures of the intraoperative insult, such as duration of cardiopulmonary bypass or ischemia.

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TABLE 1. Clinical Information

Patient	Age, y	Weight, kg	Transannular Patch	Restrictive Physiology	ICU Stay, d
1	0.58	7.6	No	Yes	10
2	0.67	8.9	No	No	2
3	1.40	9.2	Yes	No	4
4	1.40	9.4	No	Yes	14
5	1.42	8.1	Yes	No	3
6	2.72	11.7	No	No	3
7	0.64	7.7	No	No	3
8	1.73	10.6	No	No	4
9	2.98	9.4	No	Yes	8
10	1.60	9.8	No	No	3
11	3.67	14.1	Yes	Yes	8

The chronic hypoxemia of cyanotic heart disease results in a downregulation of antioxidant defenses,[7] making cells vulnerable to oxidant damage from the sudden increase in oxygen concentration at the time of surgical repair.[8,9] In vivo oxygen-derived free radical generation is critically dependent on iron being available for catalysis,[10] but normally this redox active iron is tightly sequestered in macromolecular complexes. Cyanosed patients with high hemoglobin concentrations are vulnerable to cardiopulmonary bypass–induced hemolysis that releases free hemoglobin[11] and low-molecular-weight iron, and in addition, redox active transition metals (iron and copper) are known to be mobilized after cardiac ischemia.[12] The toxicity of the superoxide radical that may be liberated by this process is potentiated by a reaction with NO to form peroxynitrite, which is deleterious in its own right but can also form the extremely damaging hydroxyl radical.[13] Even in the absence of these reactions, NO may have a negative inotropic effect on the heart.[14]

Hence, patients with tetralogy of Fallot were prospectively studied to investigate the relationship between myocardial injury, oxidative stress due to increased iron concentrations and NO production over the first 24 postoperative hours, and the subsequent development of acute RV restrictive physiology.

Methods

The protocol was approved by the Royal Brompton Hospital Clinical Research Ethics Committee, and parents of all subjects (Table 1) provided informed consent. Only patient 11 had previously received a shunt. Patients fasted for 12 hours before surgery, and only infusions of saline or dextrose solutions and human albumin replacement were given until the end of the study. No patient was given intravenous nutritional support, drug therapy, or vasoactive agents known to contain or be metabolized to nitrate compounds. Cold crystalloid cardioplegia (St Thomas' solution 1) was administered via the aorta. The mode of repair was uniform: ventricular septal defect closure was transatrial; a small right ventriculotomy was made in all patients; 3 patients required a transannular patch, and the remainder received only an outflow tract patch. Arterial samples were taken before bypass, 5 minutes after the onset of bypass, 5 minutes after removal of the cross-clamp, and 5 minutes after cardiopulmonary bypass was terminated. Subsequent sampling was at 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours after bypass. All blood samples were kept on ice, and the plasma fraction was obtained 15 minutes after sampling (4000 rpm [g_{av} 1735] at 4°C for 10 minutes).

Nitrite and Nitrate Measurement

NOx is the sum of nitrite (NO_2^-) and nitrate (NO_3^-) anions. All utensils used for the NOx samples were washed with MilliQ (pure) water before use. Plasma was stored at −70°C. NOx was measured by capillary electrophoresis (Oxonon) as NO_2^- and NO_3^-.[15]

Cardiac Troponin T

Troponin T was measured by ELISAs (ELISA troponin T, Boehringer Mannheim).[16,17]

Indexes of Iron Metabolism

The details of our measurements of iron metabolism have been reported elsewhere.[18,19] Total plasma protein was determined with a kit assay (Sigma) based on the Lowry technique. Plasma transferrin was measured by radial immunodiffusion with a polyclonal antibody to pure standards of human apotransferrin (Behring-Hoechst). Total plasma iron and iron-binding capacity were measured with a kit assay (Sigma) based on the ferrozine spectrophotometric technique. Transferrin saturation was derived from the measured total iron-binding capacity and was found to be in close agreement with values calculated from the amount of transferrin present.

Low-molecular-mass bleomycin-chelatable iron was determined as previously described.[18] Briefly, the reaction mixture contained DNA, bleomycin, and the test plasma buffered to pH 7.4 with a Tris salt. On addition of ascorbate, bleomycin chelates iron from the test plasma and degrades DNA. Malondialdehyde is released from deoxyribose and reacts with 2-thiobarbituric acid to form a chromogen that is measured spectrophotometrically.

Iron-binding antioxidant protection is an assay of plasma antioxidant activity based on the ability of transferrin to bind iron and hence inhibit iron-catalyzed free radical reactions. The iron-binding antioxidant activity of test plasma was measured in 2 different oxidizing systems, one that generated an organic oxygen radical (phospholipid peroxidation)[18] and the other an oxo-iron species (bleomycin-iron damage to DNA).[19] In both these assays, the ability of the subject's plasma to inhibit oxidation is expressed as a percentage inhibition relative to the control sample (not containing plasma) to which 100% damage occurs.

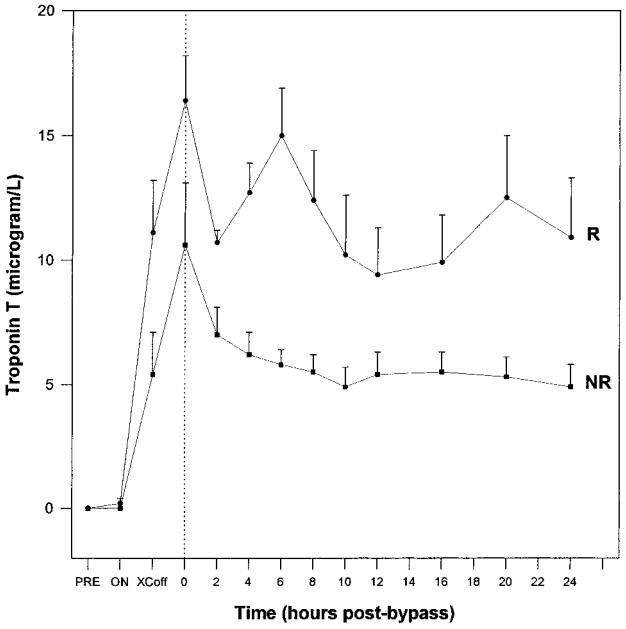

Figure 1. Arterial troponin T (mean±SE). R indicates patients with restrictive physiology; NR, patients with nonrestrictive physiology; PRE, before bypass; ON, bypass on; and XCoff, cross clamp off.

TABLE 2. Comparison of Patients With Nonrestrictive and Restrictive Physiology

	Nonrestrictive (Mean±SD)	Restrictive (Mean±SD)	*P*
Age, y	1.31±0.45	1.88±1.6	0.86
Weight, kg	9.72±1.42	10.07±3.52	0.65
Bypass duration, min	77.2±18.4	110.0±39.7	0.30
Ischemic time, min	49.8±16.5	83.3±42.5	0.23
ICU stay, d	3.0±0.63	10.7±3.1	0.007
Myocardial injury			
Arterial TnT, AUC, $\mu g \cdot h^{-1} \cdot L^{-1}$	136.2±48.3	268.8±73.6	0.02
Arterial TnT, peak, $\mu g/L$	10.4±4.6	17.0±2.8	0.03
NOx			
Creatinine, $\mu mol/L$, 24 h after bypass	60.8±14.6	61.7±18.2	1.0
NOx/creatinine, 2 h after bypass	0.8±0.2	1.3±0.4	0.04
NOx/creatinine, 24 h after bypass	0.9±0.2	1.2±0.6	0.61
Iron metabolism			
Preoperative Hb, g/dL	13.6±1.8	14.0±2.2	0.73
Total serum iron, peak, nmol/mg protein	0.55±0.2	0.69±0.18	0.22
Total serum iron, AUC, $nmol \cdot h^{-1}/mg\ protein^{-1}$	8.5±1.5	8.7±2.7	0.91
Total iron-binding capacity, minimum, nmol/mg protein	0.76±0.06	0.59±0.21	0.04
Total iron-binding capacity, AUC, $nmol \cdot h^{-1}/ \cdot mg\ protein^{-1}$	18.7±1.3	16.2±4.2	0.42
Transferrin saturation, peak, %	58.3±16.2	83.9±13.0	0.05
Transferrin saturation, AUC, % · h	1112.0±157.20	1261.5±306.31	0.33
Bleomycin-chelatable iron, n			0.36*
Iron-binding antioxidant activity (oxy-organic radicals), minimum, %	50.6±11.4	9.5±22.4	0.01
Iron-binding antioxidant activity (oxy-organic radicals), AUC, % · h	1004.5±307.9	607.0±258.7	0.06
Iron-binding antioxidant activity (oxo-iron species), minimum, %	39.3±18.3	12.3±20.1	0.10
Iron-binding antioxidant activity (oxo-iron species), AUC, % · h	1203.3±223.3	803.4±317.7	0.12

TnT indicates troponin T, and Hb, hemoglobin.
*Fisher exact test (remainder were analyzed by Mann-Whitney *U* test).

Acquisition of Echocardiograms

Echocardiograms were acquired 24 to 28 hours after surgery by use of our previously described method.[2] Briefly, imaging was performed with a Hewlett-Packard Sonos 1500 with simultaneous ECG, phonocardiogram, and respiratory motion recording. Pulmonary arterial systolic and diastolic Doppler characteristics were acquired with the pulsed Doppler sample volume placed at the midpoint between the pulmonary valve leaflets and bifurcation. Patients were divided into 2 groups, those with and those without Doppler evidence of restrictive RV diastolic physiology.

Data Analysis

Summary measures (maximum or minimum value and area under the concentration-time curve [AUC]) were used to analyze serial data, and the AUC was obtained by the trapezium rule.[20] Comparisons between the restrictive and nonrestrictive groups were by the Mann-Whitney *U* test and Fisher's exact test for proportions. The null hypothesis was rejected if $P<0.05$.

Results

Demographic data and clinical outcomes are presented in Table 1. No patient had residual RV outflow tract obstruction (Doppler gradient >30 mm Hg). There was a bimodal distribution of ICU stay, with all patients with prolonged ICU stay being characterized by the presence of restrictive RV physiology.[2] Although cardiopulmonary bypass and ischemic times tended to be longer in the restrictive group, they were not statistically different from the nonrestrictive group, and this was previously confirmed in 2 larger series of patients.[2,21]

Myocardial Injury

Arterial troponin T was undetectable before cross-clamp release, after which it rose rapidly and remained elevated for the duration of the study (Figure 1). Peak troponin T and the AUC were significantly higher in the restrictive group than in the nonrestrictive group (Table 2).

NO Metabolites

Arterial NOx fell with the onset of bypass and remained at this trough level until 6 to 10 hours after bypass, when it slowly began to rise (Figure 2). Graphic inspection showed that the restrictive and nonrestrictive groups had almost identical profiles, except that the curve for the restrictive group had a parallel upward shift, and in this small group of patients, the CIs between the 2 groups overlapped. The higher preoperative NOx concentration in the restrictive group seems largely responsible for this shift, but it remains unexplained; it was not attributable to differences in renal function, hemoglobin concentration, or degree of cyanosis. In fasted patients in the absence of exogenous NOx administration, the NOx concentration-time series reflect, albeit indi-

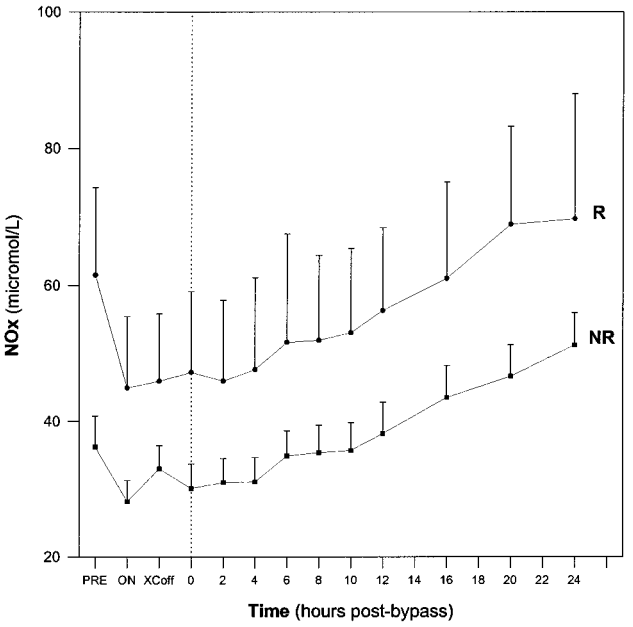

Figure 2. Arterial NOx (mean±SE). Abbreviations as in Figure 1.

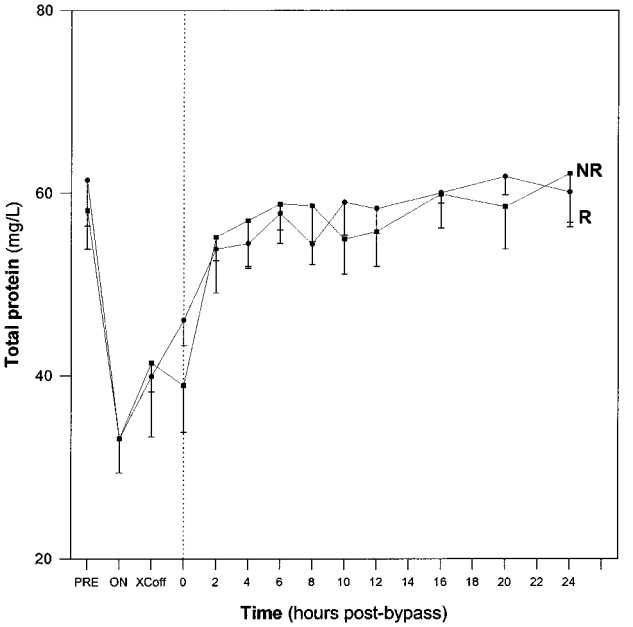

Figure 3. Arterial total plasma protein (mean±SE). Abbreviations as in Figure 1.

rectly, endogenous NOx production.[22–24] Cardiopulmonary bypass increases the extracellular fluid volume, and because the volume of distribution of NOx approximates the extracellular fluid volume,[22,23] any increase in NOx concentration implies an increase in production and/or decrease in elimination rates for NOx. NOx undergo renal elimination, and during the 2- to 24-hour postbypass period, there were only mild alterations in overall renal function, as estimated from changes in plasma creatinine, and importantly, no significant differences between the 2 groups (nonrestrictive group: 58.8±43.3%; restrictive group: 84.5±41.1%; $P=0.20$). NOx was normalized to plasma creatinine to account for differences in renal function,[25,26] and at 2 hours after bypass, the NOx/creatinine ratio (Table 2) was higher in the patients with restrictive physiology ($P=0.04$).

Iron Metabolism

The initiation of cardiopulmonary bypass produced the expected fall in total plasma protein (Figure 3) with a subsequent slow rise. There was a related fall in total iron-binding capacity (Figure 4) and transferrin concentration (not shown but analogous to Figure 3). However, whereas total protein returned to prebypass concentrations by 24 hours after bypass, the total iron-binding capacity and transferrin concentration remained depressed. Concurrent with this fall in iron-binding proteins was a rise in total serum iron, which initially rose immediately on going onto bypass but was subsequently followed by a further and greater rise at 2 to 10 hours (Figure 5). This resulted in iron loading of transferrin and elevated transferrin saturations (Figure 6) in both groups, but these were higher in the restrictive group, with the majority (3 of 4 patients) exceeding 80%, whereas this occurred in only 1 of 7 of the nonrestrictive patients (patient 3). Bleomycin-chelatable iron was detected in only 1 patient between 4 and 10 hours after bypass (Figure 7), who subsequently developed severe restrictive physiology, and did not occur in any of the nonrestrictive patients. Bleomycin cannot chelate iron from ferritin, transferrin, and heme-containing proteins, and bleomycin-chelatable iron is thought to be a low-molecular-weight iron that is catalytically active.

Plasma antioxidant activity was assayed in terms of the ability of the plasma to bind iron and inhibit formation of oxo-iron species (Figure 8) or oxyorganic radicals (Figure 9). The restrictive group had severely depressed plasma antioxidant activity compared with the nonrestrictive group, demonstrated by the minimum for the inhibition of oxo-organic radical formation (9.5±22.4%; $P=0.01$). In-

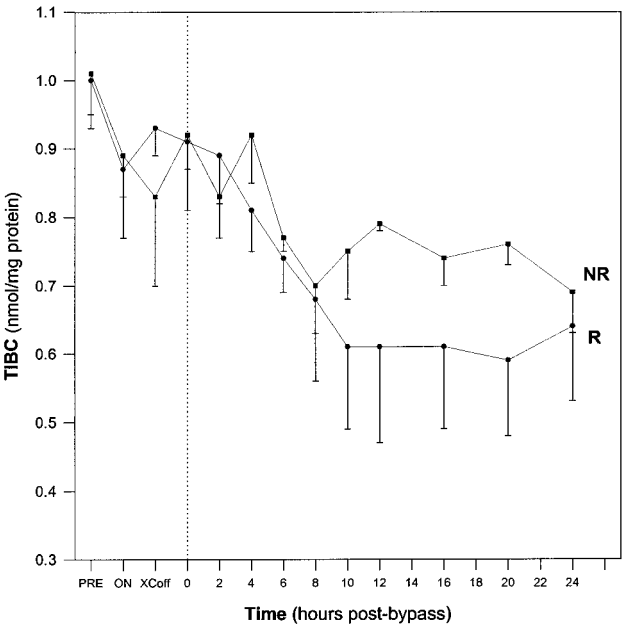

Figure 4. Arterial total iron-binding capacity (TIBC) (mean±SE). Abbreviations as in Figure 1.

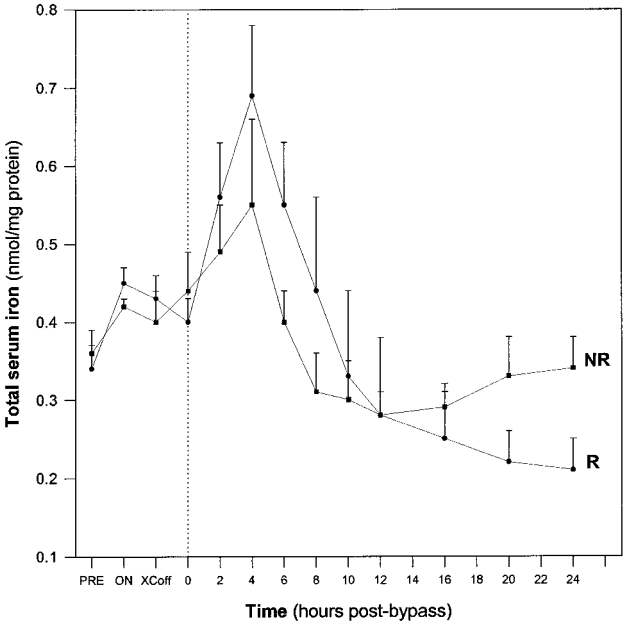

Figure 5. Arterial total serum iron (mean±SE). Abbreviations as in Figure 1.

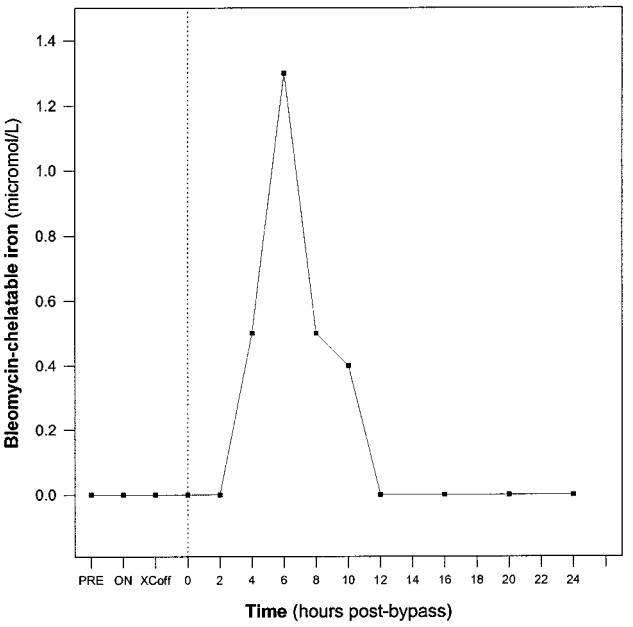

Figure 7. Arterial bleomycin-chelatable iron in patient 9 (restrictive physiology). PRE indicates before bypass; ON, bypass on; and XCoff, cross clamp off.

deed, in both these assays of iron-binding antioxidant activity, at ≥1 time point, 2 of the restrictive patients consistently had plasma that stimulated rather than inhibited oxidative reactions.

Discussion

Acute RV restrictive physiology after tetralogy of Fallot repair results in a prolonged stay in the ICU[2] and is associated with greater intraoperative myocardial injury followed by postoperative oxidative stress in the form of severe iron loading of transferrin.

Myocardial Injury

Although RV restrictive physiology is a transient phenomenon,[2,3] our patients had clear evidence of greater intraoperative myocardial injury and presumably myocyte loss. The restrictive group had both higher troponin T peak and higher AUC values than the nonrestrictive group, and indeed, the peak values in the restrictive group were in the extreme end of the range found in a previous pediatric series of patients undergoing open-heart surgery in our[16,17] and other[27] institutions. In addition, the peak troponin T values for patients with restrictive physiology were 3 to 4

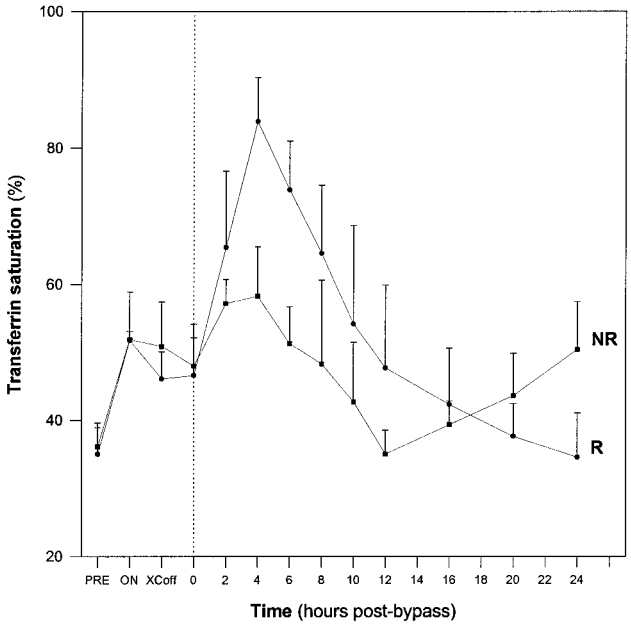

Figure 6. Arterial transferrin saturation (mean±SE). Abbreviations as in Figure 1.

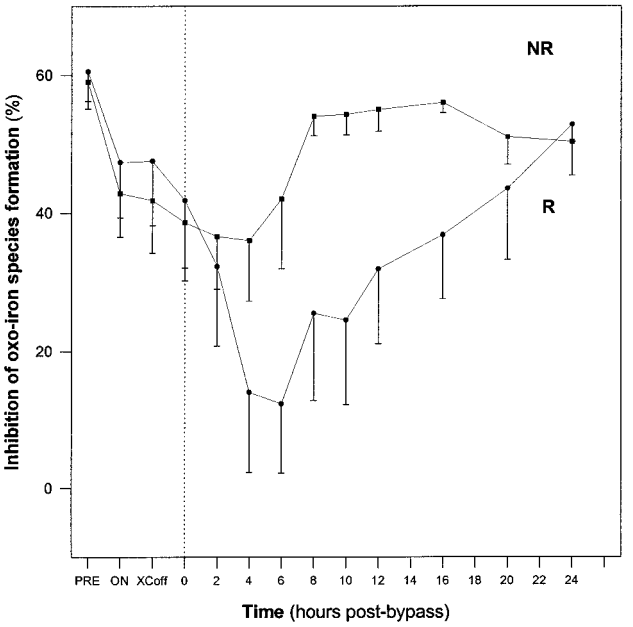

Figure 8. Arterial iron-binding antioxidant activity (oxo-iron species) (mean±SE). Abbreviations as in Figure 1.

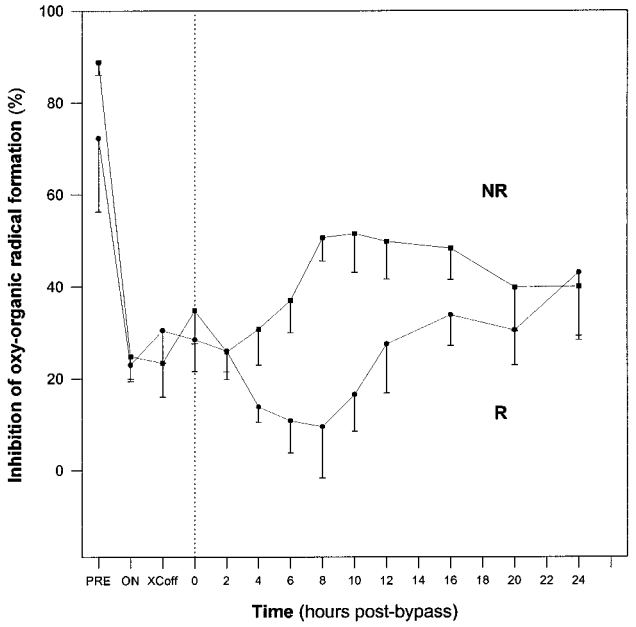

Figure 9. Arterial iron-binding antioxidant activity (oxy-organic radicals) (mean±SE). Abbreviations as in Figure 1.

times higher than previously reported values for adults undergoing coronary artery surgery[28,29] or heart transplantation[30] and lie in the upper range reported for adults with myocardial infarction.[31]

NO Metabolites

The NOx/creatinine ratio at 2 hours after bypass was higher in the restrictive group ($P=0.04$), although this difference was absent 24 hours after bypass. This may reflect higher NO levels in the early reperfusion period in the restrictive group. In addition to its direct negative inotropic effect on cardiac muscle at high concentrations,[14] NO may contribute to oxidative damage by releasing iron from ferritin[32] and exacerbating free radical–mediated injury.[33] Two previous studies[34,35] reported a perioperative NOx profile in children undergoing repair of tetralogy of Fallot that is consistent with our results. Although both had fewer tetralogy patients than the present study, NOx was found to fall with the onset of bypass, and similar to our findings, one study[34] showed there was a subsequent rise.

Iron Overload

The acute-phase response to systemic inflammation includes a fall in total iron-binding capacity and transferrin that has been attributed to extravasation of these proteins.[36] This was previously demonstrated in noncyanotic children undergoing atrial septal defect closure, a much shorter open-heart operation, in whom there was a decrease in absolute transferrin levels and a fall in transferrin saturation.[37]

Our cohort of tetralogy patients developed a pattern of iron overload superimposed on this acute-phase response. Initially with the onset of bypass, there was a fall in total plasma proteins (Figure 3), total iron-binding capacity (Figure 4), and transferrin (not shown, but analogous to Figure 3), with a raised total serum iron (Figure 5) and transferrin saturation (Figure 6). This early iron loading was followed by a more severe period of iron loading between 2 and 10 hours after bypass, characterized by elevated levels of transferrin saturation and total serum iron as well as decreased total iron-binding capacity (Table 2). The saturation of transferrin with iron in the restrictive group increased by 150% to 200%, levels that were extremely high compared with normal values in children of comparable age (restrictive group 83.9±13.0%; nonrestrictive group 58.3±16.2%; normal 5th to 95th centile range: 10% to 47%[38]). This degree of iron overload was functionally significant, because it depleted antioxidant activity in 2 assays that assessed the ability of the plasma to inhibit formation of oxo-organic radicals and oxo-iron species.[18,19] The restrictive group as a whole exhibited diminished antioxidant activity in the oxy-organic radical assay compared with the nonrestrictive group, and half of the restrictive group had frankly pro-oxidant plasma. Furthermore, 1 patient in the restrictive group had a measurable level of bleomycin-chelatable iron. This is the most severe manifestation of iron loading and represents a low-molecular-weight iron that is redox active and can function as a Fenton reagent to catalyze hydroxyl radical formation.[10] This redox active iron is particularly important because the tetralogy of Fallot myocardium has diminished antioxidant defenses and is vulnerable to free radical–mediated injury.[4,5,7] There are multiple potential sources for this iron loading, eg, bypass-related hemolysis, mobilization of tissue iron into the vascular compartment after ischemia,[12] and cardiomyocyte necrosis liberating myoglobin and other intracellular proteins containing iron.

Plasma antioxidant depletion, as measured by 2 assays different from those used in the present study, has previously been shown early after bypass in children undergoing open-heart surgery.[39] Several of these children were found to have pro-oxidant plasma, and the authors speculated that a Fenton reagent might be present, a phenomenon confirmed by our data.

Implications for Long-Term RV Function

Two syndromes of RV restrictive physiology in patients with tetralogy of Fallot have been described: an acute syndrome in the immediate postoperative period[2] and a late syndrome whose clinical manifestation is delayed by years.[40,41] Although acute RV restriction initially resolves within ≈14 days, a recent study[21] demonstrated these patients were at increased risk of subsequently developing late RV restriction and that acute RV restriction was the only independent predictor of late restriction. This late RV restriction, presumed to reflect a stiffer RV that allows less pulmonary regurgitation, has been demonstrated to result in a smaller heart, improved exercise tolerance, and decreased risk of ventricular arrhythmia.[40,41]

This study has demonstrated that during tetralogy of Fallot repair, some children experience severe myocardial injury. Peak troponin T levels in some were equivalent to those of an adult with a massive myocardial infarction, although this clearly represents more diffuse injury than that seen with coronary occlusion. Results from animal and clinical studies[42] have suggested that myocardial ischemia and infarction are followed by a reparative response that involves fibrous tissue

deposition, even at sites remote from the original lesion. Increased collagen turnover has been demonstrated in adults after myocardial infarction.[43] A similar mechanism may be present in our patient population, ie, those with the greatest intraoperative global myocardial injury subsequently develop the greatest fibrotic response, which later manifests as a noncompliant RV with echocardiographic evidence of restrictive physiology.

Conclusions

Patients destined to develop acute RV diastolic dysfunction with restrictive physiology and long ICU stays after tetralogy of Fallot repair suffer more intraoperative myocardial injury and subsequent oxidative stress related to increased iron concentrations. These observations provide novel insights into the mechanism of transient postoperative ventricular dysfunction and form the basis for future studies.

Acknowledgments

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