Ruptured abdominal aortic aneurysm, a "two-hit" ischemia/reperfusion injury: Evidence from an analysis of oxidative products

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Purpose: **Ruptured abdominal aortic aneurysm (RAAA) remains a lethal condition despite improvements in perioperative care. The consequences of RAAA are hypothesized to result from a combination of two ischemia/reperfusion events: hemorrhagic shock and lower torso ischemia. Ischemia/reperfusion results in tissue injury by diverse mechanisms, which include oxygen free radical–mediated injury produced from activated neutrophils, xanthine oxidase, and mitochondria. Oxygen-free radicals attack membrane lipids, resulting in membrane and subsequently cellular dysfunction that contributes to postoperative organ injury/failure. The purpose of this investigation was to quantify the oxidative injury that occurs as a result of the ischemia/reperfusion events in RAAAs and elective AAAs.**

Methods: **Blood samples were taken from 22 patients for elective AAA repair and from 14 patients for RAAA repair during the perioperative period. Plasma F_2-isoprostanes were extracted, purified, and measured with an enzyme immunoassay. Aldehydes and acyloins were purified and quantified. Neutrophil oxidative burst was measured in response to a receptor independent stimulus (phorbol 12-myristate 13-acetate) with luminol-based chemiluminescence.**

Results: **Plasma from patients with RAAAs showed significantly elevated F_2-isoprostane levels on arrival at hospital and were significantly elevated as compared with the levels of patients for elective repair throughout the perioperative period (two-way analysis of variance, $P < .0001$). Multiple regression showed a significant relationship between the phagocyte oxidative activity and F_2-isoprostane levels ($P < .013$). Total acyloin levels were significantly higher in patients with RAAAs as compared with the levels in elective cases.**

Conclusion: **The F_2-isoprostane levels, specific markers of lipid peroxidation, showed that patients with RAAAs had two phases of oxidative injury: before arrival at hospital and after surgery. The significant relationship between the postoperative increases in F_2-isoprostane levels and the neutrophil oxidant production implicates neutrophils in the oxidative injury that occurs after RAAA. New therapeutic interventions that attenuate neutrophil-mediated oxidant injury during reperfusion may decrease organ failure and ultimately mortality in patients with RAAAs. (J Vasc Surg 1999;30:219-28.)**

Rupture of abdominal aortic aneurysm (AAA) continues to result in significant morbidity and mortality.[1] Since the initial reports of abdominal aortic aneurysm repair, there has been a steady and progressive decline in the mortality reported for elective cases.[2,3] Katz et al[4] have noted a statewide trend for increased numbers of elective AAA repair. However, the frequency of ruptured AAAs (RAAAs) has not decreased and the mortality has remained unchanged. Population-based study results suggest that rupture of AAA is associated with a 90% mortality, yet individual centers report mortality rates that vary between 40% to 70%.[5-9] Analysis of these clinical series has determined many factors that contribute to the mortality in these patients. Rapid operative intervention without technical mishap is the current standard of therapy.[10] Postoperative treatment is directed at correction of fluid status, acid base imbalances, and hematologic

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deficiencies. However, no specific treatments have been developed to decrease the mortality from the cardiovascular events or from the multiple organ failure that frequently develops.[11] Improvements in perioperative care have not resulted in dramatic improvements in mortality.[12]

The principle difference between elective and RAAA repair is the period of hemorrhagic shock. Animal studies have established that hemorrhagic shock with resuscitation is a total body ischemia/reperfusion event.[13] All aneurysm repairs experience lower torso ischemia induced by aortic clamp application.[14] Is it possible that these two ischemia reperfusion events act synergistically in patients with RAAAs to induce the organ failure and mortality observed?

Tissue or organ ischemia, which is prolonged and followed by reperfusion, will result in organ injury and dysfunction.[15] The severity of injury is dependent on many factors, which include the degree and duration of the ischemic insult and the sensitivity of the individual tissue to ischemia. During ischemia, the lack of oxygen leads to anaerobic metabolism and depletion of energy stores, which may ultimately fail to meet the tissue energy requirements. Once the energy stores are depleted, membrane ion gradients begin to fail, membranes leak, cells swell, and the process of irreversible injury begins. Reperfusion restores tissue oxygenation but initiates an inflammatory response that has been shown to cause further tissue injury.[16-18] The xanthine/xanthine oxidase system, activated neutrophils, and mitochondrial electron transport chain leakage are documented sources of activated oxygen species during reperfusion.[19] These activated oxygen species and their metabolites (superoxide O_2^-, hydrogen peroxide H_2O_2, hypochlorus acid HOCl, and hydroxyl radicals OH^-) react with unsaturated fatty acids within the phospholipid bilayer of the cell membrane, resulting in lipid peroxidation. Oxidant-mediated injury is detected with measurement of lipid peroxidation products, such as malondialdehyde and other aldehydes, lipid hydroperoxides, conjugated dienes, and acyloins. Cleavage of the long-chain fatty acids after lipid peroxidation results in fragmentation products, which add oxygen and result in the production of aldehydes. The spectrum of aldehydes of various carbon chain lengths can be formed. However, they react rapidly and are quickly cleared from the circulation.[20] Aldehydes are detoxified by several mechanisms, including one in which compounds called acyloins are formed by the pyruvate dehydrogenase complex, resulting in the addition of a two-carbon fragment to saturated aldehydes.[21] Recently, the F_2-isoprostanes were discovered to be markers of oxidant injury.[22] These prostaglandin-like compounds are formed from free-radical catalyzed peroxidation of arachadonic acid through a noncyclooxygenase enzyme pathway.[22] These unique products can be measured and appear to be a sensitive and specific measure of free radical activity in several models of oxidant injury.[23-25] Therefore, the measurement of F_2-isoprostanes, aldehydes, and acylions will permit an assessment of oxidative lipid peroxidation in patients with RAAAs.

The purpose of this investigation was to quantify oxidative injury caused by the ischemia/reperfusion events in RAAAs and elective AAAs. Secondly, a relationship was sought between the recently identified primed neutrophil oxidative burst of patients for both elective repair and RAAA repair and the quantity of lipid oxidative products identified.

METHODS

Informed consent for blood sampling was obtained from patients for elective and RAAA repair in accordance with the Toronto Hospital and University of Toronto Human Subject Research Committee's requirements. Blood samples were harvested from the arterial line, central venous line, or a peripheral vein according to a preset sampling protocol previously described.[26] Blood samples were taken: (1) before induction of anesthesia, (2) before cross-clamp application, (3) before clamp removal, and (4) at 15, 60, and 240 minutes after clamp removal. Further samples (samples 1 to 7) were taken on a daily basis. Blood was harvested into 7-mL sterile lavender vacutainer tubes that contained 10.5 mg ethylenediamine tetraacetic acid (EDTA) and 0.014 mg potassium sorbate in 0.07 mL of 15% EDTA solution (Becton Dickinson, Franklin Lakes, NJ). The samples were kept at room temperature and were rapidly transported to the laboratory for analysis. The samples were gently rocked (Blood Rocker Model R4185-10, Baxter, Deerfield, Ill) for several minutes before an aliquot was removed for analysis of whole blood chemiluminescence and an automated complete differential blood count. The blood then was rapidly cooled to 4°C and centrifuged at 1800*g* for 15 minutes. The plasma was decanted into polypropylene tubes, snap frozen, and stored at –70°C. The aliquot of whole blood for chemiluminescence was measured at 37°C in an Autoluminat LB953 Luminometer with reagents from ExOxEmis Inc (Little Rock, Ark). Each assay was performed in triplicate with whole blood diluted 1/1000 with buffer and 7.1 μmol/L phorbol 12-

myristate 13-acetate (PMA) added to the luminol. Chemiluminescence was measured for a 30-minute period, and the area under the curve was integrated.

F_2-isoprostane measurement. Frozen plasma (0.5 mL) was thawed, and 5000 disintegrations per minute (dpm) of an internal standard (tritium labeled $PGF_{2\alpha}$) was added to quantify recovery during purification. Plasma protein were precipitated with ethanol and removed with centrifugation. F_2-isoprostanes and all other fatty acid side chains were removed from the glycerol backbone by KOH hydrolysis at 40°C. The pH was adjusted to less than 4 with HCl, and the solution was loaded onto an activated reverse phase C18 Sep-Pak Column (Waters Inc, Mississauga, Ontario). The column was washed with Ultrapure water (Millipore, Mississauga, Ontario) and hexane. F_2-isoprostanes were eluted with ethyl acetate:methanol (99:1), evaporated to dryness under dry nitrogen, and stored under nitrogen at –70°C until further analysis. The samples were analyzed in duplicate for F_2-isoprostane content with a commercially available enzyme-linked 8-isoprostane immunoassay (Cayman Chemical Co, Ann Arbor, Mich). Recovery from the purification step was analyzed with scintillation counting of the extract with Aquasol (Canberra-Packard Instruments, Meridan, Conn). The recovery range was always greater than 80%, and this was factored into the calculation. F_2-isoprostanes values are expressed as pg/mL of plasma.

Aldehyde and acylion measurement. The aldehydes and acyloins in a 100-μL plasma sample were derivitized with O-2,3,4,5,6-pentafluorobenzyl hydroxylamine hydrochloride and incubated at room temperature for 30 minutes. The protein was precipitated with methanol, and hexane was added followed by acidification with concentrated sulphuric acid. After centrifugation at 3000 rpm, the hexane layer was removed, dried over sodium sulphate, and evaporated under nitrogen. The N,O-bis(trimethylsilyl)trifluoroacetamide reagent was added followed by incubation at 80°C to form trimethylsilyl ethers. A 1-μL sample was injected into the gas chromatography mass spectrometry (GC-MS).

Capillary action column gas chromatography with negative ion chemical ionization mass spectrometry (GC-NICIMS) and ammonia as the reagent gas was used to analyze the pentafluorobenzyl-oxine-trimethylsilyl derivatives. This was performed in a high-sensitivity, research-grade quadrupole mass spectrometer (VG-Trio 2A) with an interface to a Hewlett Packard 5890 series II gas chromatograph (Mississauga, Ontario) equipped with a 30-m, 0.25-μm, DB-5 column (S & W Scientific, Folsom, Calif). The temperature of the injection port was at 250°C, and the column temperature was initially set at 60°C for 1 minute, was increased by 20°C per minute until 85°C, and then was raised 8°C per minute to 310°C. A specific ion for each derivative was chosen for selected-ion recording. The detection limits were between 50 and 100 fmol per 1 μL injected. Concentrations in the biologic samples were calculated from the ratio of peak height as compared with pure aldehydes and acyloin standards that had been injected. Results are expressed in nmol/L of plasma. This method is sensitive and completely specific for aldehyde and acyloin analysis caused by the precise identification afforded by the GC-NICIMS technology.

In vitro production of F_2-isoprostanes in activated neutrophils. Neutrophils were purified from healthy human volunteers with a percoll-hypaque gradient and differential centrifugation. The neutrophils were 95% pure by differential counting and 98% viable by trypan blue exclusion. After Coulter counter determination of the neutrophil concentration, cells were diluted to 1×10^6/mL. The neutrophils were added to an equal volume of 10% platelet-poor autologous plasma and incubated with or without PMA (100 nmol/L) for 30 minutes. PMA is a phorbol ester that stimulates neutrophil oxidative burst by diffusing across the cell membrane and directly stimulates protein kinase C without necessitating membrane receptors. The samples were rapidly cooled to 4°C and centrifuged to precipitate the neutrophils. The supernatant and neutrophils were extracted for F_2-isoprostanes content as previously described and were analyzed with the immunoassay. All the experiments were performed in duplicate, and the results were averaged. The results are expressed as pg F_2-isoprostanes/1×10^6 neutrophils and per 2 mL of supernatant.

Statistical analysis. Data were analyzed with the SAS (SAS Institute, Inc, Cary, NC) for PC statistical program, with Sigma Stat (SPSS Inc, Chicago, Ill), or with both. Continuous data were compared with univariate *t* test analysis. The main effects of group (elective vs RAAA), time (time course of measurements during and after AAA repair), and their interaction (group*time) were evaluated with two-way analysis of variance (ANOVA) for the dependent variables: F_2-isoprostanes, and neutrophil oxidant burst measured with chemiluminescence. The relationship between chemiluminescence and F_2-isoprostanes, time and group, was also evaluated with an analysis of covariance. A multiple linear regression with forward selection combined with backward elimination was performed to identify the independent predictors of F_2-

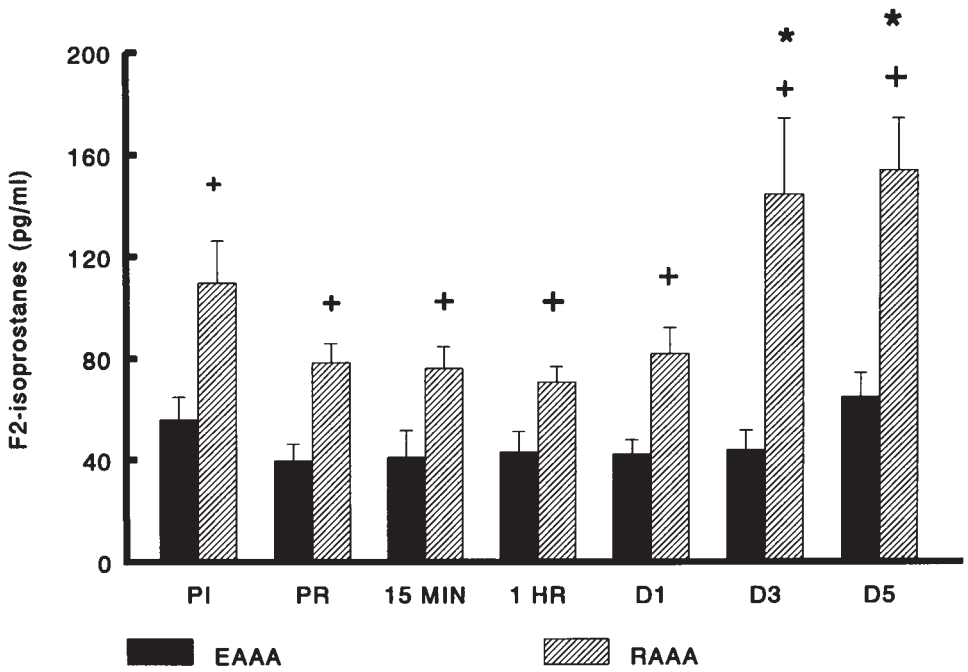

Fig 1. Plasma F_2-isoprostanes in elective and ruptured abdominal aortic aneurysm repair. Plasma levels of F_2-isoprostane measured during perioperative period for elective and ruptured aneurysms at indicated time points. Two-way analysis of variance showed significant group, time, and group*time effects.
PI, Pre induction of anesthesia; *PR*, pre release of aortic clamp; *15 MIN,* 15 minutes after clamp removal; *1 HR,* 1 hour after clamp removal; *D1*, first postoperative day; *D3,* third postoperative day; *D5,* fifth postoperative day; *EAAA,* elective abdominal aortic aneurysm repair; *RAAA,* ruptured abdominal aortic aneurysm repair. Repeated measures analysis of variance for both groups, $P < .001$.

isoprostane levels. For measures that were repeated over the time course of AAA repair, a one-way repeated measures ANOVA (RM-ANOVA) was used with comparison to the pre-induction sample (PI) with Dunnett test to correct for multiple comparisons.

The results are presented as means ± standard errors in the text, tables,and figures. The results were considered statistically significant if the *P* value was less than .05.

RESULTS

The lower torso ischemia/reperfusion necessitated for elective AAA repair did not result in any significant alteration in plasma F_2-isoprostane levels in the perioperative period. The levels of this marker of oxidant injury in these patients for elective AAA repair were slightly higher than the range reported for control groups in the literature (pre-induction 56.0 ± 8.9 pg/mL compared with literature normals 35 ± 6 pg/mL; Fig 1). This is a relatively small difference given the differences in measurement technique (radioimmunoassay vs mass spectrometry) and that our sample was of patients who underwent elective aneurysm surgery. In contrast, in patients with RAAAs, the levels of F_2-isoprostane were elevated before surgical intervention 109.5 ± 16.5 pg/mL as compared with elective 56.0 ± 8.9 pg/mL ($P < .01$, with *t* test). The levels in patients with RAAAs remained significantly elevated through the perioperative period, and further significant increases were noted on the third and fifth days after surgery in the RAAAs as compared with the preclamp release sample (109.6 ± 46.5 pg/mL vs 154.4 ± 20.3 pg/mL; $P < .05$, with RM-ANOVA, and $P < .05$, with Dunnett test; day 5). A two-way ANOVA on the combined elective and RAAA repair F_2-isoprostane data set identified that both group and time were significant main effects. The interaction term (group*time) was also significant ($P < .016$), which identifies a significantly different rate of change in

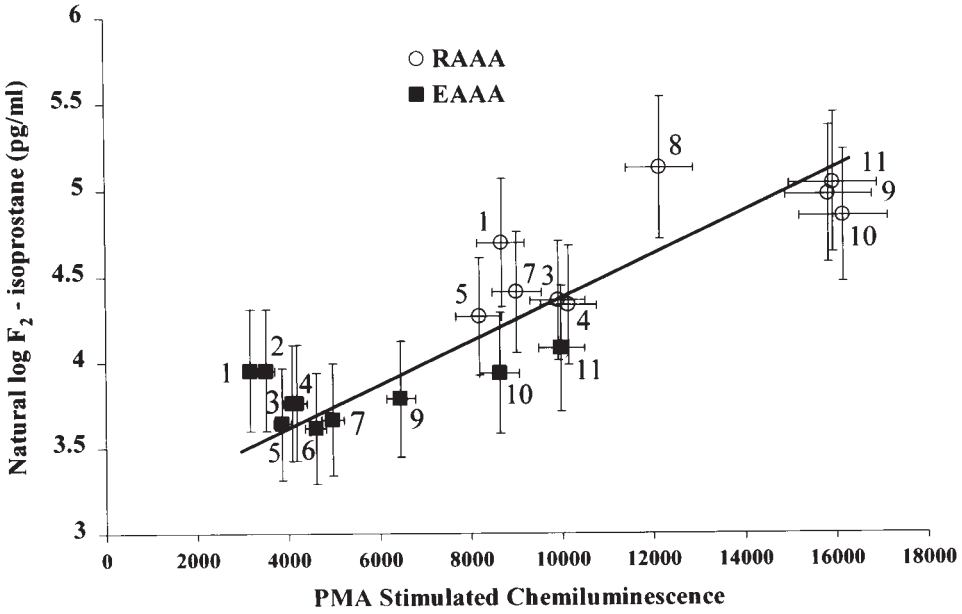

Fig 2. Relationship between stimulated neutrophil oxidative burst and index of oxidant injury over time. Linear relationship between stimulated neutrophil oxidative burst (chemiluminescence phorbol 12-myristate 13-acetate [PMA]) and level of F_2-isoprostanes measured in plasma at that time point for patients for both elective and ruptured abdominal aortic aneurysm repair. Regression line calculated for entire data is F_2-isoprostane level = 40.536 + 0.0042498 (PMA stimulated chemiluminescence). Regression line and slope for each group was significantly different. However, overall line is shown to simplify the figure. Numbers beside each data point represent time at which each sample was taken (samples 1 to 6 were taken on day of operation, and samples 7 to 11 were taken daily for first 5 postoperative days). Each point is mean of F_2-isoprostane level plotted against neutrophil oxidant burst measured by chemiluminescence.

the F_2-isoprostane levels between the two groups. An analysis of covariance with the natural log transformation of the F_2-isoprostane levels revealed a significant group and time interaction ($P < .001$), which is further statistical evidence that the slopes, or the rate of change between the two groups over time, was different ($R^2 = 0.165$; $P = .001$). We confirmed the ANOVA result with a multiple linear regression, which showed a significant association between neutrophil oxidative burst (chemiluminescence PMA), group, time, and the natural log of F_2-isoprostane ($R^2 = 0.32$; $P = .013$; Fig 2; oxidant production by neutrophils from patients with AAAs was previously published). No significant relationship was noted within each group (RAAA: $R^2 = 0.001$; $P = .71$; EAAA: $R^2 = 0.004$; $P = .36$).

Aldehyde and acylion measurements. The entire spectrum of aldehydes and acyloins was measured in both groups of patients with aneurysm. No differences in aldehyde levels between the two groups were noted (PI level, EAAA: 125.0 ± 6.4 pg/mL vs RAAA: 138.9 ± 17.1 pg/mL; at 15 minutes, 124.5 ± 8.05 vs 142.5 ± 18.6; at 1 hour, 116.2 ± 7.4 vs 117.2 ± 7.6). Fig 3 shows the alterations in the sum of all the acyloins (aldehyde detoxification products) in both groups during the early postoperative period, which were measured with GC-NICIMS. The group of elective AAA repair shows a slow rise in the total acyloin content, which became significant on the first postoperative day (RM-ANOVA, $P < .0002$; Dunnett test, $P < .05$, day 1 vs PI). In the RAAAs, the rise in acyloin level was apparent within 15 minutes of clamp release, but this was not statistically significant. By the first postoperative day, a near doubling of the total plasma acyloins was noted in the RAAA group, which was significant, as compared with the preinduction levels (RM-ANOVA, $P < .005$; Dunnett test, $P < .05$, day 1 vs PI). The patients with RAAAs had significantly higher levels of total plasma acyloin content as compared with the patients for elective repair both at 1-hour post–aortic clamp removal and at the first postoperative day.

The acyloin, 3-hydroxynonan-2-one, is derived

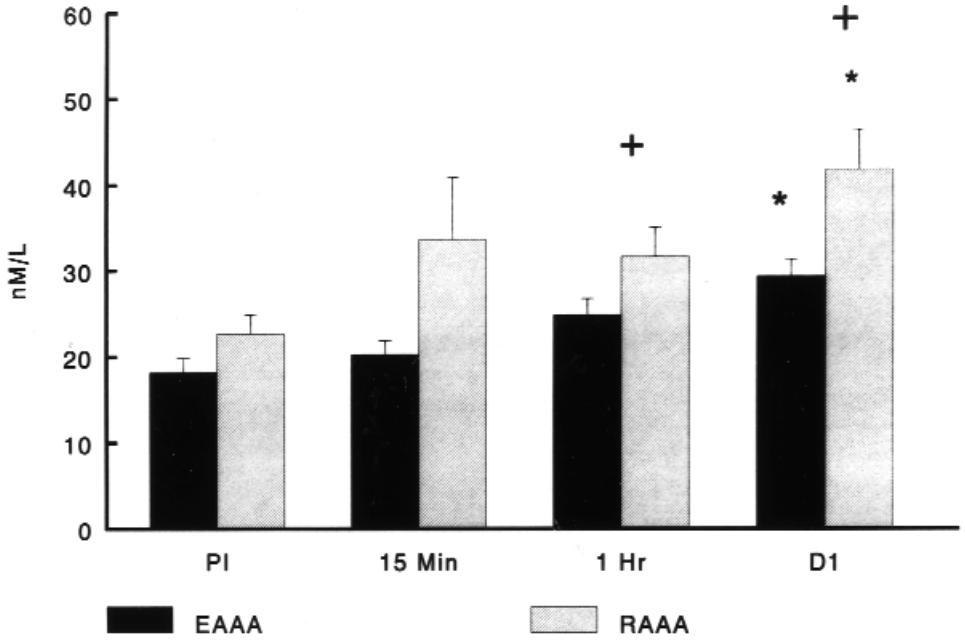

Fig 3. Total plasma acyloins. Total acyloin content of plasma is represented. Repeated measures analysis of variance for both groups, $P < .001$.
*$P < .05$ vs pre-induction sample value.
+$P < .05$ elective abdominal aortic aneurysm repair vs ruptured abdominal aortic aneurysm repair.

from the fusion of heptanal and pyruvate and shows a pattern of oxidative injury characterized by a difference noted before surgery and further progressive increases, which become significant 1 hour after aortic clamp removal (Fig 4). This acyloin (which is one of the acyloins included in the data represented in Fig 3) slowly increases in the elective AAAs, with significant increases noted at 1 hour and on the first postoperative day as compared with the preinduction level. The patients with RAAAs showed significant elevations of 3-hydroxynonan-2-one as compared with the patients for elective surgery at all time points measured ($P < .01$, RAAA vs EAAA).

In vitro production of F_2-isoprostanes by activated neutrophils. Unstimulated and stimulated neutrophils were tested in vitro to determine whether activated neutrophils resulted in increased quantities of F_2-isoprostane being produced. Neutrophils stimulated with PMA for 30 minutes significantly increased the total content of F_2-isoprostane (unstimulated 103.6 ± 4.9 pg/mL vs PMA stimulated 146.7 ± 10.3 pg/mL, $P = .001$; Table I). The increase in F_2-isoprostanes content was mainly in the neutrophil fraction of the stimulated cells (unstimulated 53.2 ± 4.3 vs PMA stimulated 78.8 ± 8.1; $P = .01$). The rise in the F_2-isoprostane content of the supernatant failed to reach significance (50.4 ± 2.6 vs 67.8 ± 7.3; $P = .14$).

DISCUSSION

This study examined two independent indices of lipid peroxidation, F_2-isoprostanes and acyloins/aldehydes, in the plasma of patients for elective and RAAA repair. Alterations in the circulating levels of these lipid peroxidation products are indices of free-radical generation and tissue injury.[27] Free radicals are generated at increased rates during reperfusion after ischemic events. Thus, identification of increases in circulating levels of lipid peroxidation products reflects the magnitude of systemic free-radical tissue injury that develops after ischemic events.

A reliable method to detect and quantify lipid peroxidation for in vitro and in vivo use has long been sought. Early measurements of lipid peroxidation were directed at malondialdehyde quantification via measurement of thiobarbituric acid reactive substances. Malondialdehyde measurements were found to lack sensitivity and specificity, and thus researchers directed their efforts at the measurement of intermediates of oxidative injury that were much

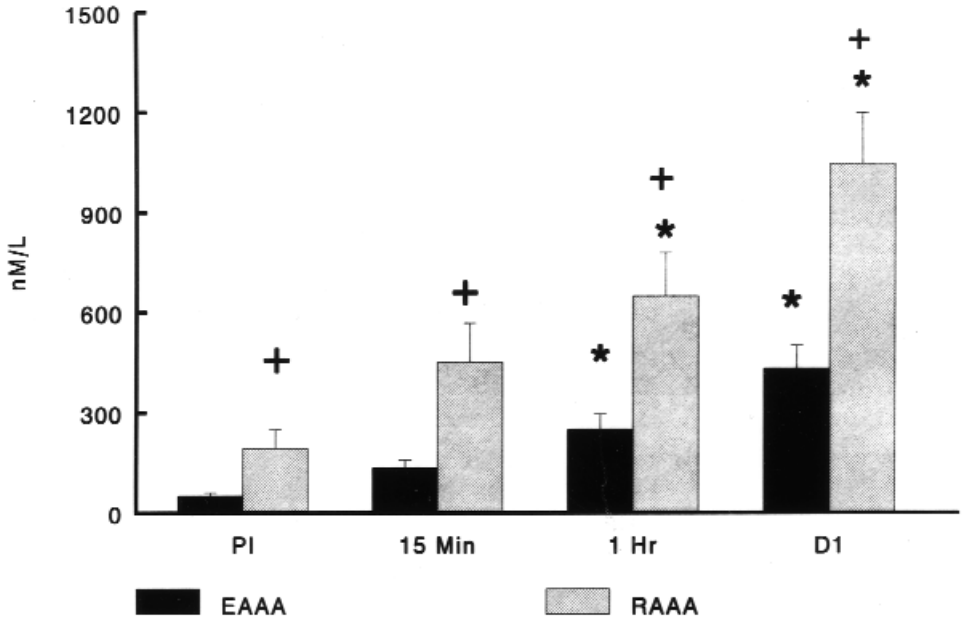

Fig 4. 3-Hydroxynona-2-one plasma levels. Repeated measures analysis of variance for both groups, $P < .001$.
*$P < .05$ vs pre-induction sample value.
+$P < .05$ elective abdominal aortic aneurysm repair vs ruptured abdominal aortic aneurysm repair.

more proximal in the chain reaction of events. Elevated conjugated dienes were measured in skeletal and cardiac muscle after ischemia/reperfusion injury that provided evidence of lipid peroxidation. However, they are technically difficult to measure.[28,29] Aldehydes are formed by lipid peroxidation and cleavage from fatty acids within cellular membranes. Recently, a method to measure all aldehydes and acyloins has been described.[20] Several groups have measured aldehyde-protein fusion products to identify and quantify peroxidation injury after ischemia/reperfusion.[21] Acyloins are derived from the addition of pyruvate to a reactive aldehyde via the pyruvate dehydrogenase complex. The heart has been shown to be a potential site of this reaction.

The measurement of F_2-isoprostanes is the most recent development in the lipid peroxidation field.[27] These products result from free radical attack on arachadonic acid.[25] This results in the formation of a stable five-member ring with striking similarity to the prostaglandin $F_{2\alpha}$ family. These compounds were initially detected and quantified with GC-MS, with detection of the negative ion chemical ionization products. The immunoassay used in this study has been shown to correlate well with the results of the GC-MS analysis. Investigations have determined that only minimal amounts of F_2-isoprostane are produced via a cyclooxygenase pathway and that the vast majority are caused by free-radical injury.[30,31] In a model of carbon tetrachloride–induced free-radical injury, F_2-isoprostanes were detected initially in the membranes of liver tissue.[32] This was followed by a progressive release of F_2-isoprostanes into the plasma, where their half life was found to be 16 minutes. Recently, increased levels of F_2-isoprostane have been identified in patients with acute myocardial infarction treated with thrombolytic therapy.[33]

Table I. The F_2-isoprostane levels from the supernatant, human neutrophil, and total content after in vitro activation of oxidant burst with PMA at 30 minutes

	Unstimulated	*PMA Stimulated*
Supernatant	50.4 ± 2.6	67.8 ± 7.3
Neutrophil	53.2 ± 4.3	78.8 ± 8.1 *
Total	103.6 ± 4.9	146.7 ± 10.3 +

PMA, Phorbol 12-myristate 13-acetate.
*$P = .01$ compared with unstimulated.
+$P = .001$ compared with unstimulated.

Animal experiments with myocardial ischemia have also shown similar results.[34] These and other experiments have established F_2-isoprostane as the current gold standard for measurement of oxidative tissue injury.

Diverse mechanisms and mediators that include neutrophils, the xanthine/xanthine oxidase enzyme system, and mitochondrial electron transport leakage cause oxidant-mediated tissue injury after ischemia reperfusion.[35] Ischemia with or without reperfusion is a central mechanism of local and remote tissue injury in many human diseases, which include myocardial infarction, stroke, embolic vascular organ or limb occlusions, trauma, and hemorrhagic shock. The detection of lipid peroxidation products implicates an oxygen-free radical mechanism of injury.

The F_2-isoprostane results show, for the first time, that the individual events that comprise repair of an RAAA (shock and lower torso ischemia) each result in significant elevations of lipid peroxidation products. The elevated levels of F_2-isoprostane noted before surgical repair in the RAAAs identifies that the "first hit" of the oxidative injury had begun before arrival at the hospital. The further significant increases noted after surgery appear to be caused by the combination of hypotension and lower torso ischemia. We noted that RAAA repair, which necessitates lower torso ischemia, induces a second phase of significant elevations in postoperative F_2-isoprostanes levels. This second rise in F_2-isoprostanes is noted on third and fifth postoperative days and is evidence of postoperative oxidative injury—"a second hit." When our results are compared with single-hit events, these injuries have a monophasic elevation in F_2-isoprostanes.[33,34] In studies of patients with myocardial ischemia, the urinary metabolites of F_2-isoprostane became elevated and fell rapidly.[33,34] Animal studies also show rapid clearance of F_2-isoprostanes from the circulation after a single oxidative event.[33] In elective AAA repairs, lower torso ischemia alone did not result in a rise in F_2-isoprostanes. Thus, the significant postoperative increases in the RAAA group suggest ongoing postoperative oxidative injury and thus provide evidence of a "two-hit" injury. Measurements of plasma acyloins, a second specific marker of lipid peroxidation, also show a larger degree of oxidative injury in the patients with RAAAs as compared with the elective repair group. Alterations in aldehyde levels may have been so transient that measurement of differences between groups was precluded because of rapid detoxification.

The significant relationship between stimulated neutrophil oxidant activity and the postoperative levels of F_2-isoprostane suggest a significant mechanistic link between these two events (Fig 2). Neutrophils are critical mediators of tissue injury after ischemia reperfusion events. However, they are by no means the exclusive cause of lipid peroxidation injury.[35,36] Although other mechanisms of oxidative injury are clearly operational, stimulated neutrophil oxidative burst is likely a significant mediator of this injury. The enhanced production of F_2-isoprostane in vitro by stimulated neutrophils shows that the oxidative burst of these cells can be responsible for production of these lipid peroxidation products. These in vitro data lend support to the mathematic relationship that we have demonstrated between the stimulated neutrophil oxidative burst and the plasma level of F_2-isoprostanes.

Overall, the oxidative injury caused by lower torso ischemia/reperfusion in patients for elective repair is modest. The elevation in postoperative acyloin levels suggests that an increase in lipid peroxidation develops early in the postoperative phase of elective AAA repair. This level of oxidative injury appears to be insufficient to increase F_2-isoprostane levels. This is in keeping with the low incidences of organ failure and mortality that follow elective AAA repair.[37]

Recently, hemorrhagic shock and resuscitation have been recognized as a whole body ischemia/reperfusion type injury.[13] A dramatic reduction in organ injury and mortality was observed when an antibody against the neutrophil CD18 adhesion receptor, which prevents firm neutrophil adhesion, was administered during resuscitation in a model of hemorrhagic shock.[38] During resuscitation from hemorrhagic shock, the no-reflow phenomenon has been documented and neutrophil adhesion in the capillaries has been described.[39] Taken together, these studies support the hypothesis that shock and resuscitation causes neutrophil activation. Also, prolonged lower torso ischemia by itself has been documented to result in local and remote organ injury.[14,40] Our results support the conclusion that each of these individual ischemia/reperfusion events results in oxidative tissue injury after repair of RAAA. The combined insult results in a systemic oxidative injury that challenges the function of every organ. Our observations suggest that therapeutic interventions directed at the oxidative injury, even if provided after the period of hypotension during the operative repair of an RAAA, may prevent further tissue injury.

In summary, this study has identified two phases of oxidative ischemia/reperfusion injury in those patients who undergo repair of RAAA with two spe-

cific and sensitive markers of lipid peroxidation. The F_2-isoprostane results suggest a "two-hit" model of injury induced by hemorrhagic shock followed by lower torso ischemia in patients with RAAAs. Neutrophils have been shown to be activated by both phases of RAAA.[26] The relationship between the F_2-isoprostane levels and the oxidant production activity of the patient's neutrophils suggests a role for neutrophil-induced oxidative tissue injury after RAAA. Treatments directed at preventing neutrophil activation or adhesion, or the scavenging of activated oxygen species, may reduce organ injury and mortality after RAAA repair.

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