Volume 120 Number 1 July 2000

The Journal of
THORACIC AND CARDIOVASCULAR SURGERY

CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

CARDIOPULMONARY BYPASS EXACERBATES OXIDATIVE STRESS BUT DOES NOT INCREASE PROINFLAMMATORY CYTOKINE RELEASE IN PATIENTS WITH DIABETES COMPARED WITH PATIENTS WITHOUT DIABETES: REGULATORY EFFECTS OF EXOGENOUS NITRIC OXIDE

Bashir M. Matata, PhD
Manuel Galiñanes, MD, PhD, FRCS

Background: Cardiopulmonary bypass induces oxidative stress and a whole-body inflammatory reaction that are believed to increase surgical morbidity.

Objectives: Our goal was to investigate the effect of nitric oxide supplementation on bypass-induced oxidative stress and inflammatory reaction in patients with and without diabetes undergoing elective coronary bypass graft surgery.

Methods: Patients with and without diabetes were randomized to receive an infusion of saline solution or the nitric oxide donor nitroglycerin at 1 μg · kg^{-1} · min^{-1} starting 10 minutes before the initiation of cardiopulmonary bypass and then maintained for 4 hours (n = 10 per group). Serial blood samples were taken at various intervals and plasma was analyzed for markers of oxidative stress (lipid hydroperoxides, protein carbonyls, and protein nitrotyrosine) and inflammation (complement C3a, elastase, interleukin 8, and tumor necrosis factor α).

Results: Cardiopulmonary bypass significantly increased lipid hydroperoxides, protein carbonyls, protein nitrotyrosine, complement C3a, elastase, soluble E-selectin, interleukin 8, and tumor necrosis factor α in both groups. Infusion of nitroglycerin significantly reduced the increase in lipid hydroper-

From the Division of Cardiac Surgery, University of Leicester, Glenfield Hospital, Leicester, United Kingdom (E-mail: mg50@le.ac.uk).

This study was partially supported by grants from The Glenfield Hospital NHS Trust, Leicester, The University of Leicester, and Heart Link Trust.

Received for publication Oct 1, 1999; revisions requested Dec 16, 1999; revisions received Jan 27, 2000; accepted for publication Feb 24, 2000.

Address for reprints: Manuel Galiñanes, MD, PhD, FRCS, Division of Cardiac Surgery, University of Leicester, Glenfield Hospital, Groby Rd, Leicester, LE3 9QP, United Kingdom.

J Thorac Cardiovasc Surg 2000;120:1-11

0022-5223/2000 $12.00 + 0 **12/1/106835**

doi:10.1067/mtc.2000.106835

oxides and protein carbonyls in patients who have diabetes without affecting levels in patients without diabetes. Nitroglycerin infusion markedly reduced protein nitrotyrosine and tumor necrosis factor α levels in both groups. In contrast, nitroglycerin infusion significantly increased C3a in patients without diabetes and increased elastase and interleukin 8 levels in patients with diabetes.

Conclusions: Cardiopulmonary bypass induces a greater oxidative stress in patients with diabetes than in those without diabetes, and the inflammatory reaction is qualitatively different in the 2 groups of patients. In addition, nitroglycerin reduces oxidative stress in patients with diabetes and differentially affects the inflammatory response to bypass both in patients with and in those without diabetes. The results have important implications with respect to the use of nitric oxide donors during cardiopulmonary bypass. (J Thorac Cardiovasc Surg 2000;120:1-11)

The use of cardiopulmonary bypass (CPB) in cardiac surgery induces a systemic inflammatory reaction and oxidative stress that is believed to result in increased postoperative morbidity and hospital stay.[1] Diabetes mellitus is an independent risk factor for early postoperative mortality and complications after bypass graft surgery,[2,3] although the reason for this heightened risk is unclear. One possibility may be the presence of a greater oxidative stress. Indeed, it has been suggested that nitric oxide (NO) and superoxide anion levels are already elevated in persons with diabetes under basal conditions.[4] The reaction between NO and superoxide anions results in the formation of the potent oxidant peroxynitrite ($ONOO^-$),[5] a factor that may exacerbate oxidative stress in patients with diabetes. Peroxynitrite is also a major source of hydroxyl radicals that are known to cause severe cellular injury and death.[6] NO donors are regularly used during and after CPB to control the patient's hemodynamics, but the effect of exogenous NO on oxidation in clinical conditions is unknown.

Another possible explanation for the greater surgical risk associated with diabetes is an altered inflammatory response to CPB. This thesis may be supported by the evidence that patients with diabetes are predisposed to increased surgical wound infections[7]; however, to the best of our knowledge, the medical literature contains no information on the subject. In this article, we speculate that exogenous NO may affect the inflammatory response to CPB since (1) inhalation of NO reduces the production of interleukin 8 (IL-8) and interleukin 6 (IL-6) in alveolar neutrophils from patients with adult respiratory distress syndrome[8] and (2) inhaled NO modulates the leukosequestration induced by tumor necrosis factor α (TNF-α) and the IL-8 release in guinea pig airways.[9]

The present study was designed to identify the type and degree of oxidative stress and inflammatory response induced by CPB in patients with diabetes compared with patients without diabetes and to investigate the effects of clinical administration of exogenous NO on these reactions.

Patients and methods

The study was carried out between 1997 and 1998 and was approved by the research ethics committee of our local health authority. Informed written consent was obtained from all participating patients.

Study groups and selection criteria. A total of 172 patients were operated on by one surgeon (M.G.) within the period of the study. Patients with ventricular aneurysm, heart failure, valvular disease, and poor left ventricular function were excluded from the study. Only 92 patients, all undergoing elective coronary bypass graft surgery, met the selection criteria, and 40 of these patients (both with and without diabetes) consented to take part in the study. The patients were blinded as to the mode of treatment, and data from all the patients were included in the final analysis. Patients (both with and without diabetes) were assigned numbers, and a computer random number generator was used to create 2 groups. One group received physiologic saline solution and the other received the NO-donor nitroglycerin (GTN) infused at a rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 4 hours, starting 10 minutes before the initiation of CPB. Administration of aspirin was discontinued at least 7 days before the operation.

Anesthesia. All the patients received morphine (10 mg) and prochlorperazine (12.5 mg) administered intramuscularly at least 1 hour before the operation. Central venous and radial artery cannulas were inserted with the aid of local anesthesia (1% lidocaine) and sedation with midazolam (3-4 mg intravenously). Anesthesia was induced by infusion of propofol (8 $mg \cdot kg^{-1} \cdot h^{-1}$ intravenously) and by the administration of fentanyl (1 mg intravenously) and pancuronium (12 mg intravenously). Anesthesia was then maintained by continu-

ous infusion of propofol (4 mg · kg^{-1} · h^{-1} intravenously). Hypotension was controlled by intravenous infusion of fluids or 0.5 mg of metaraminol with increments where appropriate. Heparin (300 IU/kg) was administered in all study patients before CPB to achieve an activated clotting time greater than 450 seconds. A further 5000 IU of heparin was given in the CPB prime for the CPB group. Heparin was reversed after CPB by the administration of protamine (3 mg/kg).

Patients were sedated with propofol (1.5-2 mg · kg^{-1} · h^{-1} intravenously) while in the intensive care unit until their core temperature was higher than 36°C and bleeding through the chest drains was less than 50 mL/h. Then if the blood P_{O_2} level was greater than 75 mm Hg and the fixed inspired oxygen fraction (F_{IO_2}) was 40% or less, the rate of propofol infusion was reduced. When the patient was able to respond coherently to simple instructions, propofol was discontinued and the trachea extubated. Morphine (5 mg intravenously) and diclofenac (100 mg intravenously), a nonsteroidal, nonnarcotic drug, were given to treat pain when required.

Surgical procedure. A standard CPB technique and median sternotomy were used. The CPB circuit was composed of a roller pump (Stöckert Instrumentation, Munich, Germany), a hollow-fiber polypropylene oxygenator with an incorporated cardiotomy reservoir (Cobe CML, Cobe Laboratories, Gloucester, United Kingdom), and plasticized polyvinyl chloride tubing. The pump was primed with 1.4 L of Hartmann solution, with a flow rate of 2.0 L · min^{-1} · m^{-2} body surface area and a hypothermic temperature of 32°C maintained throughout CPB. Coronary bypass grafting was performed with intermittent ischemia achieved by cross-clamping the aorta followed by fibrillation without the use of cold cardioplegic solution.

Blood sampling. Blood samples were collected at different times: before the induction of anesthesia, before the initiation of CPB and coronary artery clamping (time 0), and 0.5, 1, 2, 4, 8, 24, and 48 hours thereafter. Blood samples were collected into sterile tubes in 3 aliquots containing potassium ethylenediaminetetraacetic acid (EDTA). An aliquot of blood in EDTA tubes was used for total leukocyte counts. Circulating leukocytes were counted by an automatic cell counter (Cell-Dyn 610; Sequoia-Turner, Mountain View, Calif). The other aliquots were centrifuged immediately at 1500*g* for 12 minutes at 4°C. The resultant plasma was divided into aliquots and stored at –80°C until analysis.

Measurement of NO. The plasma was assayed for the stable end products of NO, nitrite and nitrate (NOx), by the Griess reaction method with kits from Cayman Chemical Co (Ann Arbor, Mich). The resultant NOx levels were corrected by plasma creatinine concentration to minimize the influence of changes in renal excretory function,[10] and the values were expressed as micromoles per millimole of creatinine. Plasma creatinine concentration was determined with a kit from Sigma Chemicals (Poole, Dorset, United Kingdom).

Assessment of oxidative stress and protein nitration. Lipid hydroperoxides, protein carbonyls, and protein nitrotyrosine levels were determined in plasma and served as indices of oxidative stress. Plasma lipid hydroperoxides[11] and protein carbonyls[12] were determined as previously described. Protein nitrotyrosine was determined by an enzyme-linked immunosorbent assay (ELISA) method also described previously.[13]

Assessment of the inflammatory reaction. The inflammatory reaction was assessed by examining the blood leukocyte count, the degree of activation of complement and neutrophils, and the plasma levels of the proinflammatory cytokines TNF-α and IL-8. Activation of complement was determined by the measurement in plasma of complement C3a by means of a sandwich ELISA (Quidel, San Diego, Calif). Activation of neutrophils was determined by the measurement of plasma elastase in complex with α_1-proteinase by means of an ELISA previously described.[14] An interassay and intra-assay variability of less than 8% was considered acceptable. The plasma levels of TNF-α and IL-8 were quantified by means of commercially available sandwich ELISAs (Pharmingen, San Diego, Calif). Soluble E-selectin and thrombomodulin in plasma were determined as markers of endothelial activation and endothelial injury, respectively,[15,16] by sandwich ELISAs for soluble E-selectin (Amersham International Inc, Amersham, United Kingdom) and thrombomodulin (Diagnostica Stago, Asniéres-Sur-Seine, France).

Clinical outcome. The time of mechanical ventilatory support, postoperative blood loss, and transfusion requirements (blood transfusion was performed when hemoglobin values fell below 10 mg/dL), the presence of postoperative fever (defined as a tympanic temperature ≥ 37.5°C and maintained for ≥ 24 hours), and the length of hospitalization were recorded. Patients were discharged from the hospital when they were apyrexic, in an overall satisfactory stable condition, and able to perform basic routine tasks.

Statistical analysis and expression of results. Descriptive data were presented as mean ± SD and nondescriptive data as mean ± SEM. Descriptive data with a skewed distribution were presented as median and quartiles. Analysis of variance for repeated measurements was used to compare the outcome parameters measured in series, that is, between-subjects effect (whether mean values vary by group), group time-response curve (whether values in groups change in different ways), and the time-trend effect (whether values change with time). This allowed us to evaluate the interaction between the CPB, diabetes, and NO effects. All the data were logarithm-transformed before analysis to minimize skewness. The data (normal distribution) determined only once during the experiment were analyzed for differences between the 2 groups by means of analysis of variance for nonrepeated measurements. The Mood median test was used to compare data measured once and also judged to be skewed. Tests were carried out at a 5% level of statistical significance with the SPSS statistical package (SPSS Inc, Chicago, Ill). Hemodilution occurs during CPB. So that we could correct for this, all values for inflammatory factors and oxidative stress markers were expressed relative to the protein content as previously described.[17]

Table I. *Patients' clinical characteristics (n = 10/group)*

	No diabetes		*Diabetes*		
	Saline solution	*GTN*	*Saline solution*	*GTN*	P *value*
Age (y)	70.1 ± 8.2	68.9 ± 11.1	65.3 ± 6.9	64.1 ± 10.8	.09
Male/female ratio	8:2	9:1	8:2	8:2	.9
Angina class (CCS)	2.7 ± 0.6	2.9 ± 0.6	3.0 ± 0.9	2.9 ± 0.6	.9
Hypertension (No. of patients)	6	4	4	5	.8
Ratio of diseased vessels (1/2/3)	0/4/6	1/0/9	0/3/7	0/1/9	.6

Data are expressed as mean ± SD. *CCS,* Canadian Cardiovascular Society; *GTN,* nitroglycerin.

Table II. *Perioperative and postoperative data (n = 10/group)*

	No diabetes		*Diabetes*		
	Saline solution	*GTN*	*Saline solution*	*GTN*	P *value*
No. of grafts/patient	2.7 ± 0.6	3.3 ± 0.9	3.0 ± 0.6	3.1 ± 0.9	.4
Ischemic time (min)	36.8 ± 13.3	44.6 ± 20.8	44.1 ± 12.0	47.4 ± 26.6	.08
CPB time (min)	81.5 ± 33.2	91.8 ± 35.1	88.2 ± 24.0	92.4 ± 35.1	.07
Ventilatory support (h)	7.1 ± 4.4	7.1 ± 3.5	8.1 ± 6.0	6.9 ± 3.2	.9
Postoperative blood loss (mL)	468 (413, 674)	595 (507, 702)	462 (301, 966)	600 (458, 1117)	.3
Blood transfusion (mL)	0 (0, 700)	350 (0, 700)	350 (0, 788)	525 (0, 1050)	.8
Postoperative fever (No. of patients)	3	5	6	4	.6
Hospital stay (d)	6.7 ± 2.2	4.5 ± 1.3	7.9 ± 5.1	5.0 ± 0.9	.08

The data for blood loss and blood transfusion were expressed as median and quartiles with statistical significance determined by the Mood median test. The rest of the data were expressed as mean ± SD. *CPB,* Cardiopulmonary bypass; *GTN,* nitroglycerin.

Table III. *Plasma NOx values (micrograms per micromole of creatinine) during and after cardiac surgery (n = 10/group)*

	No diabetes		*Diabetes*	
Time of sampling (h)	*Saline solution*	*GTN*	*Saline solution*	*GTN*
Before anesthesia	525 ± 89	440 ± 66	1011 ± 126	1265 ± 137
Before CPB (time 0)	881 ± 88	688 ± 82	1642 ± 250	1091 ± 187
0.5 h after CPB start	890 ± 108	639 ± 120	2097 ± 391	1717 ± 209
1 h after CPB start	648 ± 103	647 ± 91	3115 ± 544	1246 ± 88
2 h after CPB start	878 ± 144	578 ± 94	1686 ± 533	1040 ± 135
4 h after CPB start	814 ± 142	684 ± 101	1260 ± 110	1517 ± 228
8 h after CPB start	706 ± 96	688 ± 132	921 ± 139	1539 ± 173
24 h after CPB start	789 ± 115	840 ± 165	1163 ± 96	1697 ± 310
48 h after CPB start	527 ± 85	700 ± 134	1091 ± 148	1359 ± 172

The data were expressed as mean ± SEM. *CPB,* Cardiopulmonary bypass; *GTN,* nitroglycerin.

Results

In the groups with diabetes, the ratios of patients with insulin-dependent and non–insulin-dependent diabetes were 5:5 and 4:6 in the saline- and GTN-treated groups, respectively. There were no significant differences between patients with insulin-dependent and those with non–insulin-dependent diabetes for all the results obtained. Thus, for the sake of simplicity, the data are presented together for each of the groups.

Clinical outcome. The clinical characteristics and operative data are shown in Tables I and II. They demonstrate that these were similar in all study groups. No hospital mortality, neurologic accidents, or myocardial infarctions occurred in the 4 study groups. The mean time of mechanical ventilatory support was not significantly different among the groups, and the total postoperative blood loss, the mean values for blood transfusion, and the incidence of postoperative fever were also similar in all the groups. However, patients treated with GTN were discharged from the hospital an average of 2 days earlier than those without GTN, although the overall difference between the groups did not achieve a statistical significance ($P = .08$). A modest but significant increase in blood leukocyte count

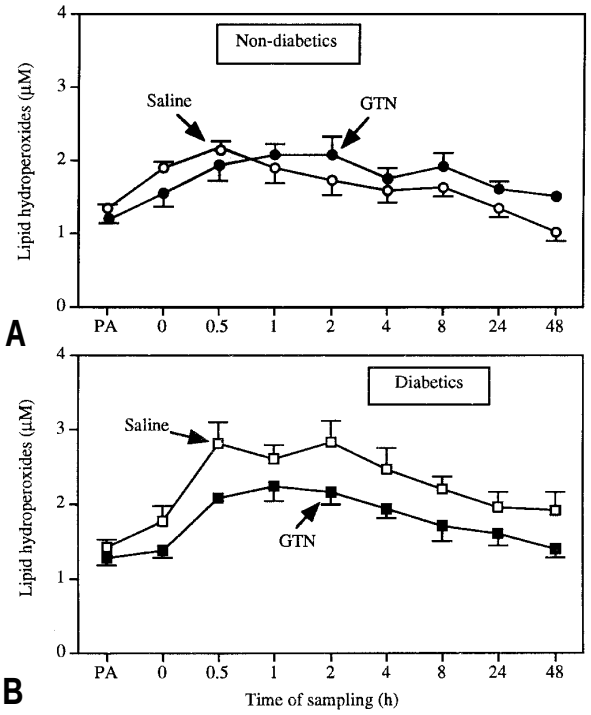

Fig 1. Plasma lipid hydroperoxides before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin *(GTN).* Values are expressed as means of 10 patients per group and *bars* represent SEM.

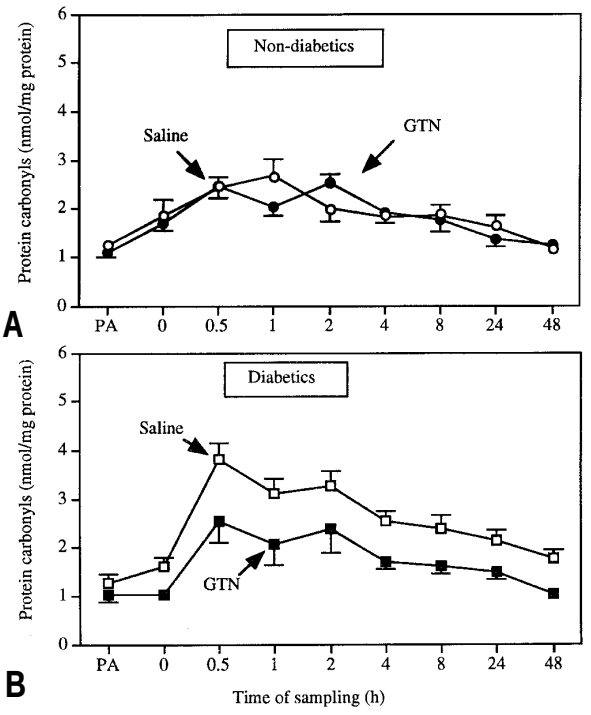

Fig 2. Plasma protein carbonyls before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin *(GTN).* Values are expressed as means of 10 patients per group and *bars* represent SEM.

was observed for the first 4 postoperative days both in patients without diabetes and in those with diabetes when compared with the values seen before anesthesia (P = .02). GTN infusion had no significant effect on leukocyte count (P = .85) in either group of patients.

Plasma NOx. As shown in Table III, the plasma mean values of NOx before anesthesia were almost 2-fold greater in patients with diabetes than in those without diabetes. Anesthesia and surgical trauma increased NOx levels in both groups (P = .01 in patients without diabetes and P = .02 in patients with diabetes); however, whereas CPB did not induce any further increase in NOx levels in patients without diabetes, values were 3-fold greater in patients with diabetes. Interestingly, GTN infusion significantly blunted the increase in NOx plasma levels in patients with diabetes (P = .003).

Oxidative stress. As shown in Fig 1, *A* and *B,* lipid hydroperoxide levels were increased by CPB and changed over time (P < .001), with mean values greater in patients with diabetes than in those without diabetes (P = .002). They also show that although GTN infusion significantly decreased mean lipid hydroperoxide levels in patients with diabetes (P = .01), it did not alter mean values in those without diabetes (P = .4). In addition, the time-response curve of lipid hydroperoxides was significantly altered by GTN infusion (P = .03) in patients with diabetes but not in those without diabetes (P = .9).

Fig 2, *A* and *B* shows that CPB induced an overall significant (P < .001) increase in the mean values of protein carbonyls in both groups of patients. A greater production of protein carbonyls was observed in patients with diabetes than in those without diabetes (P = .001). Again, GTN infusion did not alter (P = .9) the CPB-induced increase in protein carbonyls in patients without diabetes but significantly (P = .002) reduced its formation in patients with diabetes, an effect that was achieved without affecting the time-response curve (P = .8).

Fig 3, *A* and *B* shows that anesthesia and the surgical trauma increased protein nitrotyrosine formation in both groups and that further similar increases and time-course profiles were induced by CPB (P < .001). In contrast to the above results, GTN infusion significantly blunted the formation of protein nitrotyrosine in patients without diabetes (P = .03) and in patients with diabetes (P = .001).

Inflammatory factors

Complement activation. Plasma complement C3a, a product of complement activation, increased on the

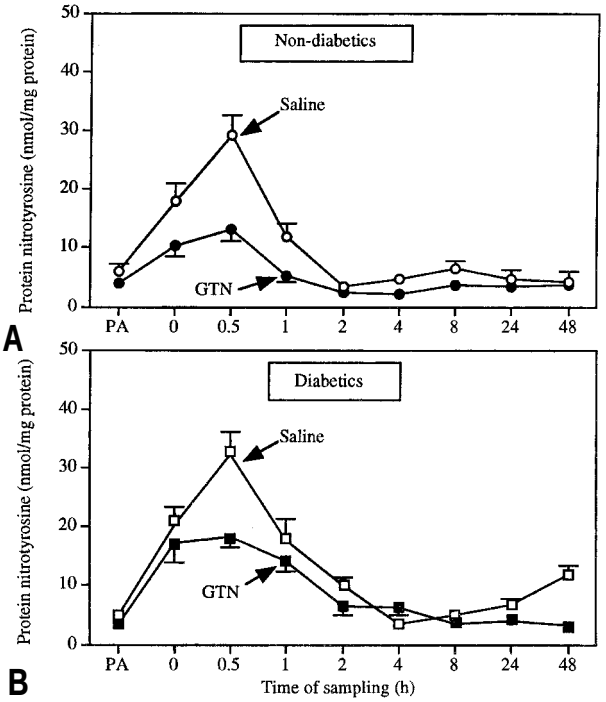

Fig 3. Plasma protein nitrotyrosine before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin *(GTN).* Values are expressed as means of 10 patients per group and *bars* represent the SEM.

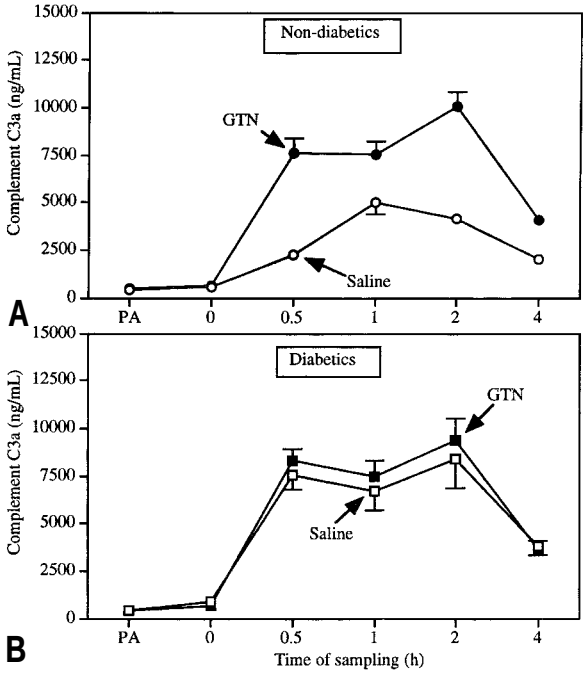

Fig 4. Plasma complement C3a before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin *(GTN).* Values are expressed as means of 10 patients per group and *bars* represent the SEM.

institution of CPB ($P < .001$) in both groups (Fig 4, *A* and *B*). However, in patients without diabetes, C3a levels increased gradually ($P < .001$) after the initiation of CPB and peaked by 1 hour, so that values were 12-fold greater than those observed before anesthesia; by contrast, in patients with diabetes, the increase was faster ($P < .001$) and greater ($P < .001$) than in patients without diabetes. Importantly, GTN infusion greatly increased C3a mean values in patients without diabetes ($P < .001$) to a degree similar to that seen in patients with diabetes, whereas its profile was unaffected in patients with diabetes ($P = .8$).

Neutrophil activation. Plasma elastase values shown in Fig 5, *A* and *B,* were significantly ($P < .001$) elevated both in patients without and in those with diabetes for up to 4 hours after the initiation of CPB. However, after this time values returned to within the preanesthesia range in patients without diabetes, whereas they remained elevated in patients with diabetes ($P = .02$). GTN infusion had no significant effect on either the mean elastase values ($P = .9$) or its time-response curve ($P = .2$) in patients without diabetes. In contrast, GTN markedly increased mean elastase values ($P = .03$) and also substantially altered the profile of the time-response curve ($P = .01$) in patients with diabetes.

Cytokines. Fig 6, *A* and *B* shows that the release of IL-8 induced by CPB was significantly greater in patients without than in patients with diabetes ($P < .001$). GTN infusion significantly reduced IL-8 mean values ($P < .001$) and altered the IL-8 time-response curve ($P = .04$) in patients without diabetes, but again, it increased IL-8 levels ($P = .004$) and altered its time-response curve ($P = .03$) in patients with diabetes.

Fig 7, *A* and *B,* shows that CPB induced a substantial increase in plasma TNF-α in patients without diabetes that lasted for the duration of the study ($P < .001$) and only a moderate but significant increase limited to the first hour in patients with diabetes ($P = .018$). The effect of GTN infusion on TNF-α release contrasts with that observed on IL-8 in that GTN completely suppressed the rise in TNF-α both in patients without diabetes ($P < .001$) and in those with diabetes ($P < .001$).

Endothelial activation. The plasma mean values of soluble E-selectin, an index of endothelial activation,

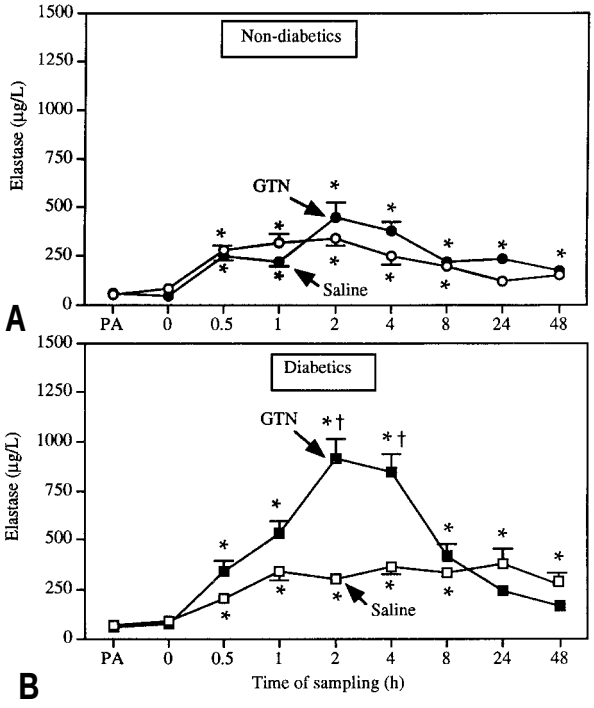

Fig 5. Plasma elastase before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin *(GTN).* Values are expressed as means of 10 patients per group and *bars* represent the SEM.

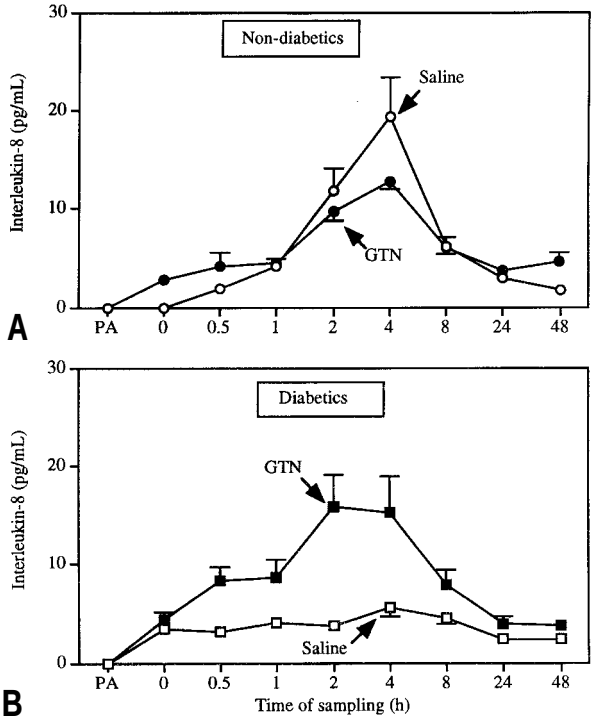

Fig 6. Plasma interleukin 8 (IL-8) before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin *(GTN).* Values are expressed as means of 10 patients per group and *bars* represent the SEM.

were modestly but significantly increased 8 hours after the initiation of CPB both in patients without and in those with diabetes (Fig 8, *A* and *B*, $P < .001$). GTN infusion had no significant effect on the mean soluble E-selectin ($P = .8$) or on the group time-response curve ($P = .13$) in patients without diabetes; in contrast, a 3-fold increase ($P = .03$) was observed by 8 hours after the initiation of CPB in patients with diabetes and significantly altered its time-response curve ($P = .01$).

Endothelial injury. Fig 9, *A* and *B* shows that the mean plasma values of thrombomodulin, an index of endothelial injury, increase with time after the initiation of CPB both in patients without diabetes and in those with diabetes ($P = .005$ in both instances) and that the release profiles are not affected by GTN infusion in the 2 groups ($P = .4$ in patients without diabetes and $P = .8$ in those with diabetes).

Discussion

This study has demonstrated that the oxidative stress induced by CPB is greater in patients with diabetes than in those without diabetes and that the inflammatory reaction is qualitatively different in the 2 groups of patients. Thus, whereas complement activation was more prominent and neutrophil activation was maintained longer in patients with diabetes than in those without, the increased production of cytokines seen in patients without diabetes was completely blunted in those with diabetes. Our study has also shown that exogenous NO significantly reduces oxidative stress in patients with diabetes and that it differentially affects the inflammatory response to CPB both in patients with and in those without diabetes. The clinical importance of these findings and their relevance to understanding the cause of oxidative stress and the regulatory mechanism of production of inflammatory factors are discussed below.

Oxidative stress. Previous studies have demonstrated that oxygen-derived free radicals are generated during CPB,[18] that lipid peroxidation occurs during CPB,[17] and that pretreatment with antioxidants before CPB minimizes lipid peroxidation.[19] However, to our knowledge, the present study is the first in reporting that CPB-induced oxidative stress is approximately 2-fold greater in patients with diabetes than in those without diabetes, a factor that may contribute to a poorer

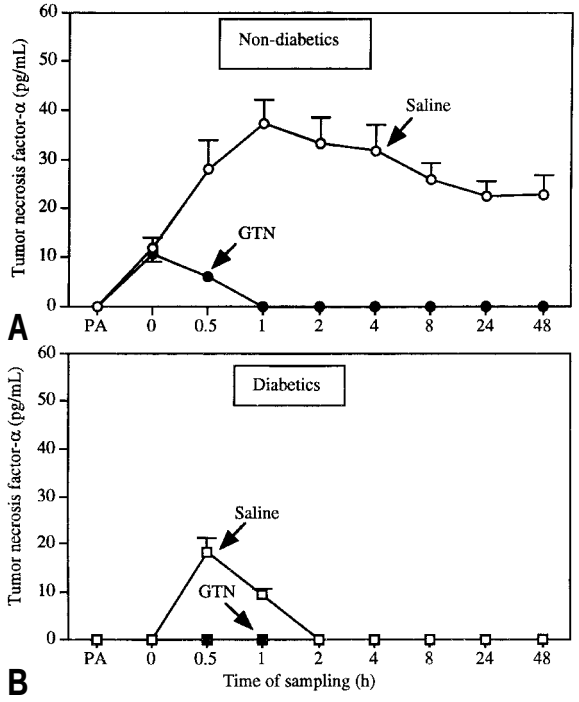

Fig 7. Plasma tumor necrosis factor-α (TNF-α) before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin *(GTN).* Values are expressed as means of 10 patients per group and *bars* represent the SEM.

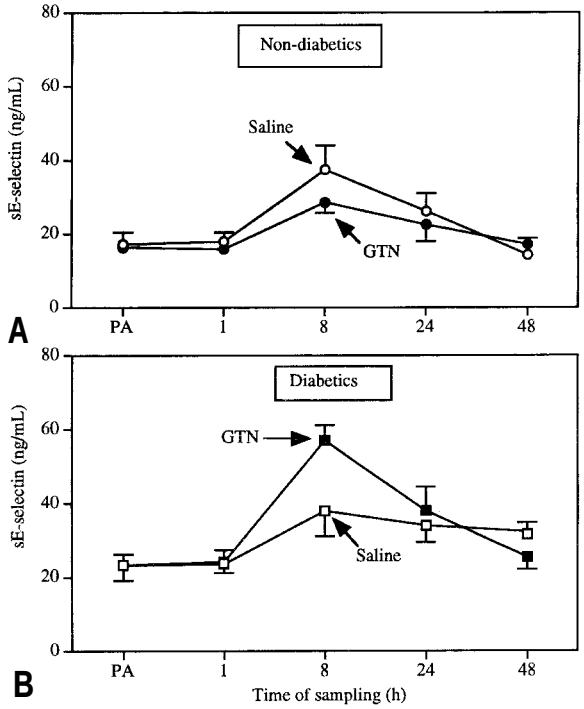

Fig 8. Plasma soluble E-selectin before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin *(GTN).* Values are expressed as means of 10 patients per group and *bars* represent the SEM.

surgical outcome in the former group of patients.[3] Yet the results were not unexpected because previous reports have suggested that persons with diabetes generate more free radicals[4] and that their antioxidant defenses are diminished.[20]

A novel finding in our study was that not only lipids but also proteins are attacked by free radicals during CPB. Our results contrast with those reported by Pepper, Mumby, and Gutteridge.[17] Like us, these authors showed formation of lipid hydroperoxides but, unlike us, they found no increase in protein carbonyls during CPB. The reason for these conflicting results is not clear, but differences in the type of patients investigated (valve replacement in their study versus coronary bypass graft surgery in ours) or the myocardial preservation techniques (intermittent ischemic arrest in this study versus cold cardioplegic arrest in their study) may offer an explanation for the differences between the 2 studies. However, both studies strengthen the view that oxidative stress may be a mechanism by which CPB exerts its undesirable effects and that antioxidant interventions may represent a potential therapeutic target.

The rapid formation of protein nitrotyrosine, an index of peroxynitrite generation, even before the initiation of CPB both in patients with and in those without diabetes (Fig 3, *A* and *B*) suggests that the metabolism of NO may play an important role in the modulation of oxidative stress observed during cardiac surgery. This thesis is further supported by the effects of GTN on decreasing the production of protein nitrotyrosine in all patients (Fig 3, *A* and *B*) and on reducing the formation of lipid hydroperoxides (Fig 1, *B*) and protein carbonyls (Fig 2, *B*) in patients with diabetes. The effects of GTN on oxidative stress may be due to the reduction in the formation of endogenous NO seen in our study (Table III) and also reported in the literature.[21] Therefore, it appears that exogenous NO has potent antioxidant properties in human beings and that its clinical usefulness may be extended beyond the control of the hemodynamic state.

Inflammatory reaction. The increase in CPB-induced inflammatory reaction in terms of complement and neutrophil activation in patients with diabetes may be a contributory factor to the reported higher operative mortality and greater rate of perioper-

ative complications in this group of patients undergoing cardiac surgery.[3] The cause of this reaction and the pathophysiologic implication of the lack of increase in proinflammatory cytokines in patients with diabetes are unclear. A possible explanation may be that the inflammatory reaction is initiated or facilitated by oxidative stress. At first sight this thesis would appear to be supported by our findings that oxidative stress is greater in patients with diabetes than in those without diabetes and by the reported correlation between complement activation and oxidative stress during CPB.[18] However, in our study the reduction in the CPB-induced oxidative stress by GTN in patients with diabetes resulted in a partially corresponding decrease in the CPB-induced inflammatory response. On the contrary, GTN increased plasma elastase (Fig 5, *B*) and IL-8 (Fig 6, *B*), without affecting complement activation (Fig 4, *B*) and TNF-α (Fig 7, *B*). Therefore, results do in fact contradict the thesis that there is a direct relationship between the CPB-induced inflammatory reaction and oxidative stress.

An alternative explanation for the specific inflammatory reaction induced by CPB in patients with diabetes is that the regulation of the genes coding for a variety of inflammatory factors and their signal transduction pathways may be altered in this group of patients. This proposition is supported by the demonstration that protein kinase C is chronically activated in diabetic tissues,[22,23] which in turn may activate the stress/mitogen protein kinase–associated transcription factor activator protein–1.[24] Our demonstration of higher complement activation (Fig 4, *B*) with prolonged release of elastase (Fig 5, *B*) in patients with diabetes would also support this thesis. However, the validity of this thesis has to be questioned in view of our findings that the release of IL-8 and TNF-α was lower in patients with diabetes. The diversity of these results may be owed to the involvement of different gene transcription factors and signal transduction mechanisms and to the fact that these may be differently affected in patients who do and do not have diabetes. This is an area that clearly requires further investigation.

The results of the administration of exogenous NO on the CPB-induced release of inflammatory factors seen in the present study are also novel. The observed increase in complement activation in patients without diabetes (Fig 4, *A*) and the higher plasma elastase (Fig 5, *B*) and IL-8 (Fig 6, *B*) in patients with diabetes when GTN was administered are indicative that exogenous NO exacerbates the CPB-induced inflammatory reaction. Since NO is known to possess anti-inflammatory properties, the action of exogenous NO in promoting inflammation may be explained by its effect in reducing

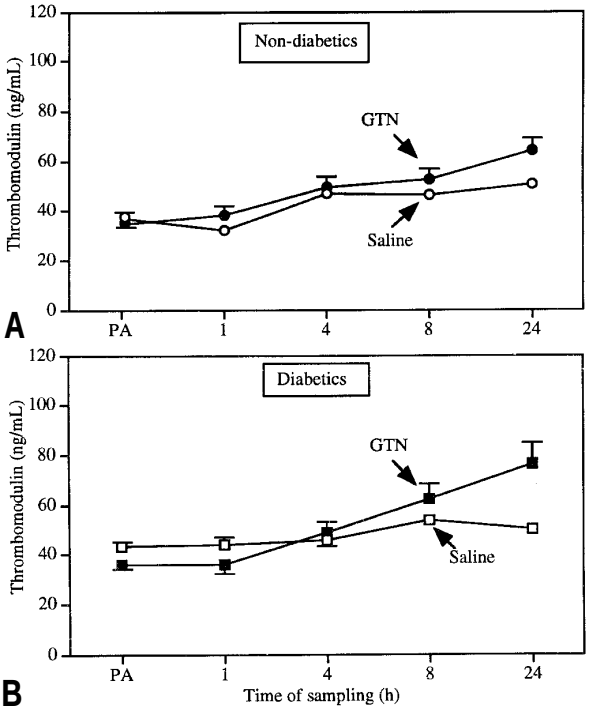

Fig 9. Plasma thrombomodulin before, during, and after coronary bypass graft surgery in patients without diabetes **(A)** and in those with diabetes **(B)** receiving saline solution or nitroglycerin (*GTN*). Values are expressed as means of 10 patients per group and *bars* represent the SEM.

the formation of endogenous NO, as mentioned before. Our results contrast with those of Seghaye and colleagues,[25] who observed the inhibition of complement activation by sodium nitroprusside. However, in contrast to our study, these investigators used sodium nitroprusside instead of GTN. In addition, their study was carried out in children with congenital heart defects instead of adults with coronary artery disease, and a sodium nitroprusside dose ranging from 0.6 to 2.9 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was variably administered only during the cooling and rewarming periods of extracorporeal circulation. In our study, GTN was administered at the dose of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ starting 10 minutes before the initiation of CPB and then for the next 4 hours, which included the entire period of CPB. Indeed, the type of NO donor and the time and dose of its administration may be important factors in determining the effect of these agents on the inflammatory cascade.

In the present study, GTN also reduced the formation of TNF-α in patients without diabetes (Fig 7, *A*), and this may suggest that the effect of exogenous NO on inflammation is complex and dependent on the disease state. The literature is also conflicting in that NO has

been shown to inhibit[26] and activate[27,28] the production of inflammatory cytokines. The understanding of this effect is crucial to elucidate the dynamics and the clinical potential of NO. Clearly, this topic also requires further investigation.

The results on soluble E-selectin, an endothelial molecule that facilitates the recruitment of monocytes and lymphocytes to the lesion site,[29] showing that its plasma values remained elevated for a longer period in patients with diabetes than in those without diabetes (Fig 8, *A*), are in agreement with our own data that the inflammatory reaction induced by CPB is exacerbated in patients with diabetes. However, at this point it could also be speculated that the release of soluble E-selectin into the plasma compartment may not be indicative of parallel changes in the density and expression of the molecule on the endothelium; instead, it may represent a protective mechanism that allows the clearance of these molecules from the cell surfaces, therefore limiting leukocyte attachment and cell injury.[30] As seen with plasma elastase and IL-8, GTN also increased soluble E-selectin in patients with diabetes (Fig 8, *B*). At first sight, one might be tempted to conclude that the effect in patients with diabetes would be proinflammatory. However, using the above argument, if the elevation of soluble E-selectin in plasma represents an increase of the shedding of the molecule and not an increase of its expression, then it should be concluded that the effect of GTN is anti-inflammatory. Indeed, the latter thesis is supported by the demonstration that shedding of molecules from the endothelium may be influenced by a number of factors,[30] possibly including NO, as shown by our own results. As discussed earlier, whether this effect is due to the exogenous NO or to the inhibition of production of endogenous NO by exogenous NO also needs to be clarified. De Caterina and coworkers[26] reported that NO significantly inhibits the expression of vascular cell adhesion molecule 1 and soluble E-selectin in lipopolysaccharide and cytokine-stimulated human saphenous vein endothelial cells in vitro. However, their findings do not completely elucidate the mechanism of this effect, because the expression and activity of endothelial and inducible NO synthases and the levels of NO in the preparation were not studied.

Despite the different response to CPB and the infusion of GTN between patients with and without diabetes, in terms of both oxidative stress and inflammatory reaction, plasma thrombomodulin, a marker of endothelial injury,[16] was similar in the 2 groups of patients and this was unaffected by GTN (Fig 9, *A* and *B*). These results appear to strengthen the thesis that the vascular endothelium is well equipped to withstand the changes occurring during elective cardiac surgery and that disruption of its integrity may require more severe challenges.

Clinical implications. Our results have shown that oxidative stress is greater in patients with diabetes than in those without diabetes and that its reduction by NO-donor agents may represent a potential target to improve the clinical outcome in this group of patients undergoing cardiac surgery. Our study, performed in a selected reduced number of patients, was not designed to investigate the clinical benefit of this therapy. However, despite this, a shorter hospital stay (marginally not significant) was seen in patients receiving GTN. It should be conceded that the shorter hospital stay was also observed in patients without diabetes, in whom oxidative stress was unaffected by GTN, an observation that would suggest that the action of GTN in reducing oxidative stress may not be the sole mechanism responsible for the improved recovery.

This study has also demonstrated that GTN may increase the release of some components of the inflammatory reaction induced by CPB both in patients with diabetes and in those without diabetes. As a result and despite the observed benefits in reducing oxidative stress in patients with diabetes, caution should be exercised regarding the use of NO donors, particularly when treating patients with an underlying proinflammatory condition. Certainly, the elucidation of the mechanisms underlying the above effects is a prerequisite for safer use of NO donors and for improvement of the clinical outcome in cardiac surgery.

We acknowledge Dr David Duthie and Dr J. Swanevelder (Department of Anesthesia, Glenfield Hospital) for the help with the anesthesia of patients. We acknowledge Mr Nick Taub (Department of Epidemiology and Public Health) and Mrs Yana Podinovskaia (Division of Cardiology, University of Leicester) for the advice on statistical analysis. We also acknowledge Dr A. Fowler (Cardiac Surgery, Glenfield Hospital) for his help in preparing this manuscript.

REFERENCES

1. Butler J, Rocker GM, Westaby S. Inflammatory response to cardiopulmonary bypass. Ann Thorac Surg 1993;55:552-9.
2. Cohen Y, Raz I, Merin G, Mozes B. Comparison of factors associated with 30-day mortality after coronary artery bypass grafting in patients with versus without diabetes mellitus. Am J Cardiol 1998;81:7-11.
3. Fietsam RJ, Bassett J, Glover JL. Complications of coronary artery surgery in diabetic patients. Am Surg 1996;57:551-7.
4. Cosentino F, Hishikawa K, Katusic ZS, Lüscher TF. High glucose increases nitric oxide synthase expression and superoxide anion

generation in human aortic endothelial cells. Circulation 1997;96:25-8.

5. Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin JC, Smith CD, et al. Peroxynitrite-mediated tyrosine nitration catalysed by superoxide dismutase. Arch Biochem Biophys 1992;298:431-7.
6. Beckman JS, Beckman TW, Chen PA, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 1990;87:1620-4.
7. Vuorisalo S, Haukipuro K, Pokela R, Syrjala H. Risk features for surgical-site infections in coronary artery bypass surgery. Infect Control Hosp Epidemiol 1998;19:229-33.
8. Cholet-Martin S, Gatecel C, Kermarrec N, Gougerot-Pocidalo MA, Payen DM. Alveolar neutrophils functions and cytokine levels in patients with ARDS during nitric oxide inhalation. Am J Respir Crit Care Med 1996;153:985-90.
9. Kuo HP, Hwang KH, Lin HC, Wang CH, Lu LC. Effect of endogenous nitric oxide on TNF-α-induced leukosequestration and IL-8 in guinea-pigs airways in vivo. Br J Pharmacol 1997;122:103-11.
10. Böger RH, Bode-Böger SM, Gerecke U, Gutzki FM, Tsikas D, Frölisch JC. Urinary NO_3^- excretion as an indicator of nitric oxide formation in vivo during oral administration of L-arginine or L-NAME in rats. Clin Exp Pharmacol Physiol 1996;23:11-5.
11. Nourooz-Zadeh J, Tjaddini-Sarmadi J, Wolff SP. Measurements of plasma lipid hydroperoxide concentrations by ferrous oxidation–xylenol orange assay in conjunction with triphenylphosphine. Anal Biochem 1994;220:403-9.
12. Buss H, Chan TP, Sluis KB, Domigan NM, Winterbourn C. Protein carbonyl measurement by a sensitive ELISA method. Free Radic Biol Med 1997;23:361-6.
13. Khan J, Brennan DM, Bradley N, Gao B, Bruckdorfer R, Jacobs M. 3-Nitrotyrosine in the proteins of human plasma determined by an ELISA method. Biochem J 1998;300(Pt 2):795-801.
14. Neumann S, Jochum M. Elastase-α1-proteinase inhibitor complex. In: Bergermeyer HU, Bergermeyer J, Grassl M, editors. Methods of enzymatic analysis 5. Weinheim: Verlag Chemie; 1984. p. 184-95.
15. Blann AD, Seigneur M, Steiner M, Boisseau MR, McCollum CN. Circulating endothelial cell markers in peripheral vascular disease: relationship to the location and extent of atherosclerotic disease. Eur J Clin Invest 1997;27:916-21.
16. Komai H, Haworth SG. Thrombomodulin and angiotensin-converting enzyme activity during pediatric open heart operations. Ann Thorac Surg 1996;62:553-8.
17. Pepper JR, Mumby S, Gutteridge JMC. Sequential oxidative damage and changes in iron-binding and iron-oxidising plasma antioxidants during cardiopulmonary bypass surgery. Free Radic Res 1994;21:377-85.
18. Cavarocchi NC, England MD, Schaff HV, Russo P, Orszulak TA, Schnell WA Jr, et al. Oxygen free radical generation during cardiopulmonary bypass: correlation with complement activation. Circulation 1986;74(Pt 2):III-130-3.
19. England MD, Cavarocchi NC, O'Brien JF, Solis E, Pluth JR, Orszulak TA, et al. Influence of antioxidants (mannitol and allopurinol) on oxygen free radical generation during and after cardiopulmonary bypass. Circulation 1986;74(Pt 2):III-134-7.
20. Nourooz-Zadeh J, Rahimi A, Tajaddini-Sarmadi J, Tritschler H, Rosen P, Halliwell B, et al. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologia 1997;40:647-53.
21. Ravichandran LV, Johns RA, Rengasamy A. Direct and reversible inhibition of endothelial nitric oxide synthase by nitric oxide. Am J Physiol 1995;268:H2216-23.
22. Inoguchi T, Battan R, Handler E, Sprotman JR, Heath W, King GL. Preferential elevation of protein kinase C isoform βII and diacylglycerol levels in the aorta and heart of diabetic rats: differential reversibility to glycemic control by islet cell transplantation. Proc Natl Acad Sci U S A 1992;89:11059-63.
23. Lee TS, Saltsman KA, Ohashi H, King GL. Activation of protein kinase C by elevation of glucose concentration: proposal for a mechanism in the development of diabetic vascular complications. Proc Natl Acad Sci U S A 1989;86:5141-5.
24. Gopalakrishna R, Chen Z, Gundimeda U. Nitric oxide and nitric oxide–generating agents induce a reversible activation of protein kinase C activity and phorbol ester binding. J Biol Chem 1993;268:27180-5.
25. Seghaye MC, Duchateau J, Graitz RG, Wolff T, Marcus C, Engelhardt W, et al. Effect of sodium nitroprusside on complement activation induced by cardiopulmonary bypass: clinical and experimental study. J Thorac Cardiovasc Surg 1996;111:882-92.
26. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Ghimbrone MA Jr, et al. Nitric oxide decreases cytokine-induced endothelial activation: nitric oxide selectively reduces endothelial expression of adhesion molecules and pro-inflammatory cytokines. J Clin Invest 1995;96:60-8.
27. Meldrum DR, Shames BD, Meng X, Fullerton DA, McIntyre RC Jr, Grover FL, et al. Nitric oxide downregulates lung macrophage inflammatory cytokine production. Ann Thorac Surg 1998;66:313-7.
28. Deakin AM, Payne AN, Whittle BJ, Moncada S. The modulation of IL-6 and TNF-alpha release by nitric oxide following stimulation of J774 cells with LPS and IFN-gamma. Cytokine 1995;7:408-16.
29. Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA Jr. Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci U S A 1987;84:9238-42.
30. Wyble CW, Hynes KL, Kuchibhotla J, Marcus BC, Hallahan D, Gewertz BL. TNF-alpha and IL-1 upregulate membrane-bound and soluble E-selectin through a common pathway. J Surg Res 1997;73:107-12.