Effect of Diabetes on Nitric Oxide Metabolism During Cardiac Surgery

Bashir M. Matata and Manuel Galiñanes

The metabolism of nitric oxide (NO) during cardiac surgery is unclear. We studied the effect of diabetes on NO metabolism during cardiac surgery in 40 subjects (20 with diabetes and 20 without diabetes). The patients were randomized to receive an infusion of physiological saline or nitroglycerin (GTN) at 1 μg · kg^{-1} · min^{-1} starting 10 min before the initiation of cardiopulmonary bypass and then continuing for a period of 4 h. Blood and urine samples were collected at several time points for up to 8 h. NO metabolites were determined by the measurement of nitrate/nitrite (NOx, μmol/mmol creatinine) and cyclic guanosine monophosphate (cGMP, nmol/mmol creatinine) in plasma and urine. Plasma insulin levels were also determined at selected time points. Plasma NOx levels before surgery were significantly elevated in the group with diabetes compared with the group without diabetes ($P < 0.001$), and values were further increased during surgery in the former ($P = 0.005$) but not in the latter ($P = 0.8$). The greater plasma NOx values in patients with diabetes were matched by commensurate elevations in plasma cGMP levels ($P = 0.01$). Interestingly, infusion of GTN, an NO donor, significantly reduced plasma NOx ($P < 0.001$) and its urine elimination ($P < 0.001$) in patients with diabetes without reducing plasma cGMP levels ($P = 0.89$). Cardiac surgery increased plasma insulin in patients with and without diabetes; this increase was delayed by the infusion of GTN, but it was not related to the changes in NO production. In conclusion, NO production during cardiac surgery is increased in patients with diabetes, and this elevation can be blunted by the infusion of GTN in a rapid and reversible manner. *Diabetes* 50:2603–2610, 2001

Diabetes is recognized as a major independent risk factor for cardiovascular disease (1). In addition, patients with diabetes undergoing cardiac surgery have a greater rate of perioperative complications and increased mortality than those without diabetes (2,3), but the reason for this is unknown. Diabetes is associated with altered endothelial vascular responses (4), and modifications in nitric oxide (NO) metabolism may play a role. This thesis finds support in the observation that diabetes affects basal NO metabolism (5). We have also recently demonstrated (6) that the provision of exogenous NO during cardiac surgery influences oxidative stress and the inflammatory response to cardiopulmonary bypass (CPB), effects that are more prominent in patients with diabetes than in those without diabetes. NO, synthesized by NO synthase (NOS) from a terminal guanidino group of L-arginine (7), relaxes vascular smooth muscle and inhibits platelet aggregation and adhesion via the second messenger cyclic guanosine monophosphate (cGMP) (8), and it has also been implicated in the pathophysiology of a number of cardiovascular diseases, including atherosclerosis (9), septic shock (10), inflammation (11), oxidative stress (12), and ischemia/reperfusion injury (13). NO is rapidly oxidized to nitrate and nitrite (14), and it is subsequently eliminated by excretion into the urine (15). Recent studies have shown that there is an uptake of NO into the erythrocytes and that its conversion to nitrate/nitrite (NOx) and methemoglobin is a major pathway of degradation for endogenously formed NO (16). NOx may then enter the plasma to be eliminated via the kidneys. However, it is unclear how NO metabolism may be affected by diabetes and cardiac surgery.

The metabolism of NO is rapid, and this may pose difficulties for the assessment of NO production. To overcome this, a possibility may be to determine the stable NO metabolites NOx and the second messenger cGMP in plasma and in urine, as described by Wennmalm et al. (16), with values corrected by creatinine clearance to take into account changes in renal excretory function, as advocated by Böger et al. (17). In the present study, using this approach, we assessed NOx and cGMP values in plasma and urine to investigate the effect of diabetes on the metabolism of NO during cardiac surgery and determine whether this is affected by the administration of the NO donor nitroglycerin (GTN). Because insulin increases NOS expression (18) and NO production (19–21), insulin plasma levels were also determined.

RESEARCH DESIGN AND METHODS

Study groups and selection criteria. The study was conducted in accordance with the principles expressed in the Declaration of Helsinki, and local ethical committee approval was obtained. Informed written consent was authorized by all participating individuals after the nature of the investigation was explained to them. A total of 170 patients were operated on by one surgeon (M.G.) during a 12-month period. Individuals with valvular disease, ventricular aneurysm, peripheral vascular disease, carotid stenosis, heart failure, and poor left ventricular function were excluded from the study. Only 86 patients, all undergoing elective coronary bypass graft surgery, met the selection criteria, and 40 of these patients (20 with diabetes and 20 without diabetes) consented to take part in the study. The patients were blinded as to the mode of treatment, and data from all of the patients taking part in the study were included in the final analysis. Patients taking part in the study

From the Division of Cardiac Surgery, Department of Surgery, University of Leicester, Glenfield Hospital, Leicester, United Kingdom.

Address correspondence and reprint requests to Manuel Galiñanes, MD, Division of Cardiac Surgery, Department of Surgery, University of Leicester, Glenfield Hospital, Leicester, LE3 9QP, U.K. E-mail: mg50@le.ac.uk.

Received for publication 26 June 2000 and accepted in revised form 10 August 2001.

AUC, area under the curve; cGMP, cyclic guanosine monophosphate; CPB, cardiopulmonary bypass; GTN, nitroglycerin; NO, nitric oxide; NOS, NO synthase; NOx, nitrate/nitrite.

(both with or without diabetes) were assigned numbers, and a computer random number generator was used to create two groups. One group received physiological saline solution, and the other received the NO donor GTN, which was infused at a rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ starting 10 min before the initiation of CPB and then continued for 4 h.

Before surgery, blood glucose levels were well controlled by either insulin ($n = 10$) or oral antidiabetic therapy ($n = 10$) in all patients, as shown by the HbA_1 values (range 7.2–9.7% with a mean value of 8.5 ± 0.3% compared with the normal 4–8.5% normal range). Patients with diabetes attended the Diabetes Clinic in the Department of Endocrinology at the University Hospital of Leicester, Leicester General Hospital, for treatment. The disease classification was undertaken in accordance with the revised American Diabetes Association (22) and the World Health Organization criteria (23). During the study, the infusion of insulin to control blood glucose commenced immediately after the termination of CPB in all patients with diabetes and was maintained for 48–72 h. Blood glucose levels were monitored periodically, and they were kept under control by a sliding scale regime for insulin administration using a stock concentration of 1 IU/ml insulin (Actrapid) suspended in normal saline. When blood glucose was 0–4, 4–6, 6–8, 8–10, 10–12, 12–14, and >14 mmol/l, patients were given a corresponding insulin infusion of 0, 2, 4, 6, 8, 10, or 12 ml/h, respectively. Hemoglobin levels were also monitored periodically, and blood transfusion was performed to maintain values >10 mg/dl.

Blood and urine sampling. Arterial blood samples were collected at the following time points: before the induction of anesthesia, before the initiation of CPB (time 0), and 0.5, 1, 2, 4, and 8 h thereafter. Samples were collected into sterile EDTA tubes and centrifuged immediately at 1,500*g* for 12 min at 4°C. The resultant plasma was aliquoted and stored at −80°C until analysis.

Immediately before the induction of anesthesia, a urine catheter (Cobe CML; Cobe Laboratories, Gloucester, U.K.) was inserted into the bladder and connected to a reservoir bag. The initial urine samples were discarded, and samples for analysis were collected at time 0–1, 1–2, 2–4, and 4–8 h after the initiation of CPB and immediately snap frozen and stored at −80°C until analysis.

Anesthesia. The administration of anesthesia was in accordance with institutional guidelines. All of the volunteers received morphine (10.0 mg) and prochlorperazine (12.5 mg) administered intramuscularly at least 1 h before surgery. Central venous and radial artery cannulae were inserted under local anesthesia (1% lidocaine) and with midazolam sedation (3.0–4.0 mg i.v.). Anesthesia was induced with an infusion of propofol (8.0 $mg \cdot kg^{-1} \cdot h^{-1}$ i.v.) and with fentanyl (1.0 mg i.v.) and pancuronium (12.0 mg i.v.). Anesthesia was then maintained by continuous infusion of propofol (4.0 $mg \cdot kg^{-1} \cdot h^{-1}$ i.v.). Hypotension was controlled by intravenous infusion of fluids or 0.5 mg metaraminol, with increments where appropriate. Heparin (300 IU/kg body wt) was administered in all study patients just before aortic cross-clamping to achieve an activated clotting time of >450 s. A further 5,000 IU heparin were given in the bypass prime. Morphine (5.0 mg i.v.) and diclofenac (100.0 mg i.v.), a nonsteroidal nonnarcotic drug, were given to treat pain when required and if not contraindicated.

Surgery. After the induction of anesthesia and the systemic application of heparin, CPB was initiated in a standard fashion under conditions of mild body hypothermia (32°C). Briefly, CPB was composed of a roller pump circuit (Stöckert Instrumente, Munich, Germany), a hollow fiber polypropylene oxygenator with an incorporated cardiotomy reservoir (Cobe CML), and plasticized polyvinyl chloride tubing. The pump was primed with 1.4 l Hartmann's solution and used at a flow rate of 2.4 $l \cdot min^{-1} \cdot m^{-2}$ body surface area. The coronary bypass grafts were performed using intermittent periods of ischemia without using cardioplegic solutions. Heparin was reversed after bypass by the administration of 3.0 mg/kg protamine.

Measurement of NOx. Plasma and urine samples were assayed for NOx, the stable end product of NO, by using a commercially available kit (Cayman Chemical, Ann Arbor, MI). Briefly, the plasma and urine samples were first filtered through a 0.45-μm Minisart column (Amersham, U.K.). The filtrate was incubated with nitrate reductase and NADH as an enzyme cofactor for 3 h at room temperature to reduce nitrate to nitrite. The total nitrite (NOx) was then analyzed by reacting the samples with Griess reagent and measuring the absorbance of each sample at 540 nm. The amounts of NOx in plasma and urine were calculated from a known concentration range of a standard curve obtained by enzymatic conversion of sodium nitrate to sodium nitrite. To rule out a potential interfering effect of GTN on the measurement of NOx in plasma, pooled plasma obtained from 10 healthy subjects was spiked with 100 μmol/l nitrate. In the presence and absence of 10 μmol/l GTN, the assay detected 116 and 110 μmol/l of NOx in the plasma, respectively, indicating a lack of effect of GTN on the assay. The intra- and interassay coefficient of variation of the NOx assay in plasma was 9.0 and 9.2%, respectively. The detection limit of the NOx assay was 2.5 μmol/l, and regression coefficients for the standard curves ranged between 0.98 and 0.999.

Measurement of cGMP. Plasma and urine cGMP were measured by a competitive enzyme immunoassay kit (Cayman Chemical). In plasma, cGMP was at first extracted after precipitation with cold ethanol. After centrifugation at 1,500*g* for 10 min, the resultant supernatant was collected into clean test tubes. The supernatant was dried under a stream of nitrogen before resuspension in phosphate buffer. Urine samples were diluted 100 times with phosphate buffer and centrifuged at 2,500*g* at 4°C for 10 min. To enhance the sensitivity of the assay, all of the samples and standards were acetylated before the assay by the addition of 4 mol/l potassium hydroxide followed by acetic anhydride (5:1 ratio).

The assay is based on the competition between free cGMP and a cGMP tracer (cGMP linked to an acetylcholinesterase molecule) for a limited number of cGMP-specific rabbit antiserum binding sites. Thus, the amount of cGMP tracer that is able to bind to the rabbit antiserum is inversely proportional to the concentration of free cGMP in plate wells. A constant tracer concentration was maintained, whereas the concentration of free cGMP (standard and samples) was varied. The resultant rabbit antiserum–cGMP complex (either free or tracer) binds to a mouse monoclonal anti-rabbit antibody that has been previously attached to the plate wells after 18 h incubation at room temperature. After washing to remove any unbound reagents, Ellman's reagent (containing the substrate to acetylcholinesterase) was added to the wells. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The color intensity was proportional to the amount of cGMP tracer bound to the well, which in turn was inversely proportional to the free cGMP present in the well during the incubation. As before, to investigate a potential interfering effect of GTN on the measurement of cGMP in plasma, pooled plasma obtained from 10 healthy subjects was spiked with 100 pmol/ml cGMP. In the extracted supernatants, the assay detected 94 and 92 pmol/ml of cGMP in the presence and absence of 10 μmol/l GTN, respectively, indicating a lack of effect of GTN on the assay. The detection limit of the cGMP assay was 0.09 pmol/ml and the intra- and interassay coefficient of variation was 9 and 9.7%, respectively.

Measurement of creatinine. To minimize the influence of changes in renal excretory function, NOx and cGMP values were corrected for creatinine concentration. Creatinine concentration was determined by a colorimetric assay from Sigma Chemicals (Poole, Dorset, U.K.). The assay was based on the reaction between creatinine and alkaline picrate, with a resulting yellow/orange color. Under acid conditions creatinine-picrate color fades faster than interfering chromogens. Therefore, measurement of the difference in color intensity at or near 500 nm before and after acidification is proportional to creatinine concentration. The resultant plasma content and urinary excretion of NOx and cGMP were corrected by creatinine concentration expressed as micromoles per millimole and picomoles per millimoles creatinine, respectively, as described previously (16,17). Creatinine and nitrate clearances were calculated as follows: clearance = urine flow (ml/min) × urinary concentration (μmol/l) ÷ plasma concentration (μmol/l). Pooled plasma with mean creatinine levels of 1.1–6.9 mg/dl assayed by this method on 10 separate occasions produced standard deviations of 0.12–0.25 mg/dl and coefficients of variance of 10.9–3.6%.

Measurement of plasma insulin. Blood glucose was determined by a glucose-oxidase method (Unimate 5 Glucose HK; Roche, Basel, Switzerland) using a Vitros 250 autoanalyzer (Orthoclinical Diagnostics, Buckinghamshire, U.K.). Plasma insulin was determined by an enzyme-linked immunosorbent assay for human insulin (DRG International, Mountainside, NJ). The minimum detectable concentration of insulin estimated by this assay was 0.15 μIU/ml. The intra- and interassay coefficient of variance ranged from 3 to 5.3 and 5.6 to 9.8%, respectively. There was no evidence of cross-reactivity with proinsulin measurement in this assay.

Statistical analysis. All of the data are presented as the means ± SE of means in plots of values against time in hours (Figs. 1–5). For the purpose of statistical comparisons, each parameter value plot against time in hours from the start of the study for each subject, and the area under the curve (AUC) was calculated as the primary measure of outcome. There was evidence of positive skewness in some of the raw data-time points, and for this reason logarithm transformation was used to ensure nearly equal variability in the groups being compared. The main analysis is a two-way analysis of variance of the AUC (calculated on the log10 scale) to assess the apparent effect on outcome by GTN, diabetes, and any corresponding interaction. The results were presented as the difference between groups in the mean value of AUC, with the corresponding 95% CIs, and P values ≤5% were considered to be of statistical significance. A secondary analysis was then carried out to validate the statistical significance of these results using the program Proc Mixed in the computer package SAS to fit an autocorrelation for repeated measures. The correlation of any two measurements made on the same subject was assumed

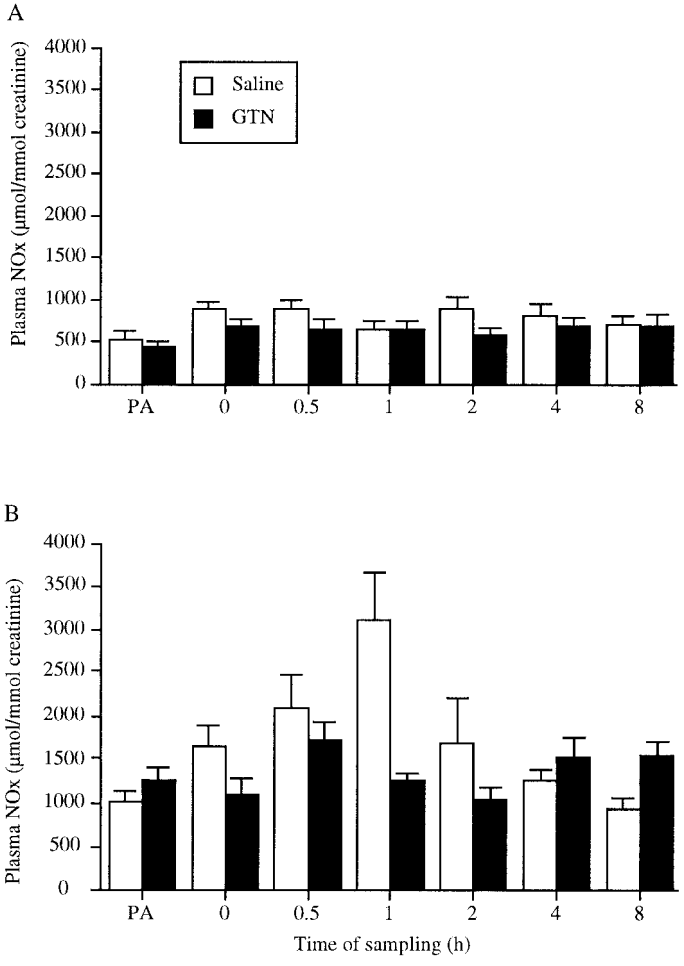

FIG. 1. Changes in plasma NOx before (PA, before anesthesia), during (first 2 h of sampling), and after cardiac surgery in patients without diabetes (*A*) and with diabetes (*B*) receiving an infusion of saline or GTN at a rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ starting 10 min before the institution of CPB and then maintained for a period of 4 h after the initiation of CPB. Values were expressed as the arithmetic mean of 10 patients per group, and bars represent the SE.

to be ρ^d, where d is the intervening period of time and ρ is common to all subjects.

RESULTS

Demographic data. As shown in Table 1, the clinical characteristics and perioperative data were similar in all study groups. Table 2 shows that the medication administered to patients before surgery was also similar in all study groups, with the exception of the antidiabetic treatment. All of the patients with diabetes had the type 2 form of the disease. The results were similar for the patients with diabetes regardless of whether they were on insulin and, because of this, the values are presented together without distinction of the treatment for the control of diabetes.

NOx

In plasma. The results shown in Fig. 1*A* and *B* demonstrate that the NOx mean plasma values at baseline (i.e., preanesthesia) were twofold greater in patients with diabetes compared with those without diabetes ($P < 0.001$). They also show that NOx values were not significantly affected by cardiac surgery (e.g., the first 2 h of sampling) in patients without diabetes, whereas they were significantly ($P = 0.02$) elevated in patients with diabetes. Interestingly, the infusion of GTN significantly ($P = 0.003$) reduced plasma NOx only in patients with diabetes.

In urine. Figure 2*A* and *B* shows that the urinary excretion of NOx was greater in patients with diabetes compared with those without diabetes during the first hour of surgery ($P = 0.01$), although the overall urinary excretion of NOx over the duration of the study was similar in both study groups ($P = 0.83$). GTN infusion, however, significantly reduced urinary NOx in both groups ($P = 0.005$ in the group with diabetes and $P = 0.002$ in the group without diabetes).

Plasma creatinine clearance. Table 3 shows the results for plasma creatinine clearance, which are presented as ratios of geometric means of AUCs over the entire study period. The results show that plasma creatinine clearance is similar in the two groups ($P = 0.5$) and that the infusion of GTN had no significant effect on creatinine clearance in both groups of patients ($P = 0.9$ in the group with diabetes and $P = 0.14$ in the group without diabetes).

Plasma NOx clearance. Table 3 also shows the results with regard to plasma NOx clearance. Plasma NOx clearance was found to be influenced by diabetes, with values significantly lower ($P = 0.002$) in patients with diabetes than in those without diabetes. On the other hand, GTN significantly reduced ($P = 0.02$) plasma NOx clearance in patients with diabetes but had no significant effect on patients without diabetes ($P = 0.3$). These results, taken together with the unaffected urinary NOx excretion and plasma creatinine clearance shown above, suggest that the increase in plasma NOx in patients with diabetes represents a real increase in NO production that is not readily eliminated by the kidneys.

cGMP

In plasma. Figure 3*A* and *B* shows that, in contrast with the higher levels in plasma NOx in patients with diabetes, cGMP plasma mean values were similar in the two patient groups before anesthesia. They also show that as with

TABLE 1
Clinical characteristics and perioperative data

	Without diabetes		With diabetes		
	Saline	GTN	Saline	GTN	*P*
Age (years)	70.1 ± 2.6	68.9 ± 3.5	65.3 ± 2.2	64.1 ± 3.8	0.13
M/F	8/2	9/1	8/2	8/2	0.92
Angina class (CCS)	2.7 ± 0.2	2.9 ± 0.2	3.0 ± 0.3	2.9 ± 0.2	0.64
No. of grafts per subject	2.7 ± 0.2	3.3 ± 0.3	3.0 ± 0.2	3.1 ± 0.3	0.35
Ischemia time (min)	36.8 ± 4.2	44.6 ± 6.6	44.1 ± 3.8	47.8 ± 8.4	0.14
Bypass time (min)	81.5 ± 10.5	91.8 ± 11.1	88.2 ± 7.6	92.4 ± 11.1	0.08

Data are arithmetic means ± SE. CCS, Canadian Cardiovascular Society.

TABLE 2
Preoperative medication

	Without diabetes		With diabetes		
	Saline	GTN	Saline	GTN	*P*
Nitrates	8	7	8	8	0.94
β-Blockers	8	5	6	6	0.59
ACE inhibitors	5	3	3	6	0.46
K_{ATP} channel openers	2	3	1	0	0.29
Ca^{2+} channel antagonists	10	6	9	8	0.11
Insulin	0	0	4	6	<0.01
Oral antidiabetics	0	0	6	4	<0.01

In each group, $n = 10$ patients. K_{ATP}, ATP-sensitive K^+ channel.

NOx, the effect of cardiac surgery on cGMP values was not statistically significant in patients without diabetes, but that it caused a significant increase in the early phase of surgery in patients with diabetes (47 ± 6 vs. 102 ± 12 μmol/mmol creatinine, respectively; $P = 0.005$). However, the ratios of geometric means of AUC for the overall plasma cGMP values were not significantly different ($P = 0.3$) between the two groups during the entire study period. Importantly, GTN caused a significant increase in the AUC mean values for plasma cGMP in patients without diabetes ($P < 0.001$), whereas it had no significant ($P = 0.89$) effect in patients with diabetes.

In urine. As a reflection of the results on plasma cGMP, Fig. 4*A* and *B* shows that surgery increased urinary cGMP excretion between 1 and 4 h in patients with diabetes, whereas it had no marked effect in the group without diabetes. In addition, ratios of the AUC for the entire study period further indicate that urinary cGMP excretion was significantly greater ($P = 0.02$) in the group with diabetes (3,854 ± 17) compared with the group without diabetes (2,612 ± 13). These figures also show that the infusion of GTN resulted in an early increase in cGMP excretion in the two study groups ($P = 0.001$). This was followed by a period of no further significant increase in both study groups ($P = 0.5$ in the group with diabetes and $P = 0.9$ in the group without diabetes).

Plasma cGMP clearance. The results shown in Table 3 indicate that the mean AUC for cGMP clearance was significantly greater ($P = 0.05$) in patients with diabetes compared with those without diabetes. In addition, GTN infusion significantly reduced cGMP clearance in both study groups ($P = 0.04$ and $P = 0.05$, respectively), an effect that may be responsible, at least in part, for the plasma cGMP elevation seen in patients without diabetes.

TABLE 3
Ratios of geometric means for AUC with the corresponding 95% CIs for creatinine, NOx, and cGMP clearances

	Saline	GTN	*P*
Without diabetes			
Creatinine	812 (678–974)	588 (416–834)	0.14
NOx	74 (59–92)	58 (33–102)	0.31
cGMP	2,344 (1,542–3,563)	724 (209–2,506)	0.04
With diabetes			
Creatinine	776 (597–1,009)	832 (479–1,445)	0.9
NOx	38 (27–53)*	24 (19–31)	0.02
cGMP	3,715 (1,531–9,016)	1,919 (714–4,188)	0.05

Data are means (95% CI). *$P = 0.002$ versus the corresponding mean values in the group without diabetes.

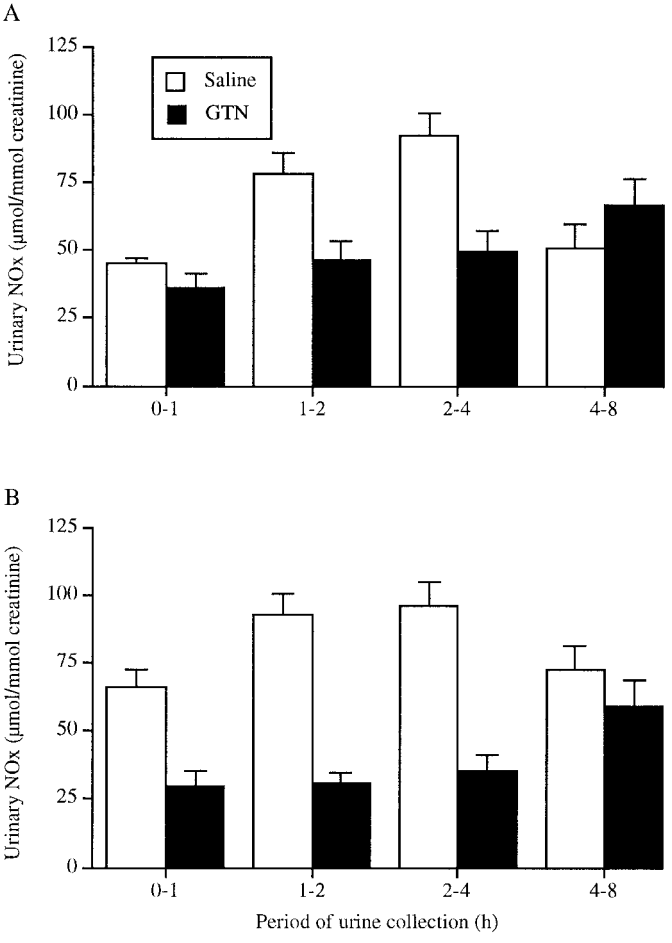

FIG. 2. Changes in urinary NOx excretion during (first 2 h of sampling) and after cardiac surgery in patients without diabetes (*A*) and with diabetes (*B*) receiving an infusion of saline or GTN at a rate of 1 μg · kg^{-1} · min^{-1} starting 10 min before the institution of CPB and then maintained for a period of 4 h after the initiation of CPB. Values were expressed as the arithmetic mean of 10 patients per group, and bars represent the SE.

Plasma insulin. Figure 5*A* and *B* shows that cardiac surgery induces a significant increase in plasma insulin in patients without diabetes and a still greater increase in those with diabetes that peaked at 1 h after the initiation of CPB. Interestingly, this rise in plasma insulin was delayed but not suppressed by the infusion of GTN in both groups of patients. There was no further significant increase in insulin levels after the administration of exogenous insulin.

Blood glucose. As seen in Table 4, despite the changes in plasma insulin, blood glucose levels remained within the same range throughout the entire study period in both groups of patients; however, values were more elevated in patients with diabetes than in patients without diabetes.

DISCUSSION

The present study has shown for the first time that cardiac surgery markedly affects the NO metabolism in patients with diabetes. Although plasma NOx values were already significantly elevated before surgery in the group with diabetes as compared with those without diabetes, further increases occurred during surgery in the former but not in

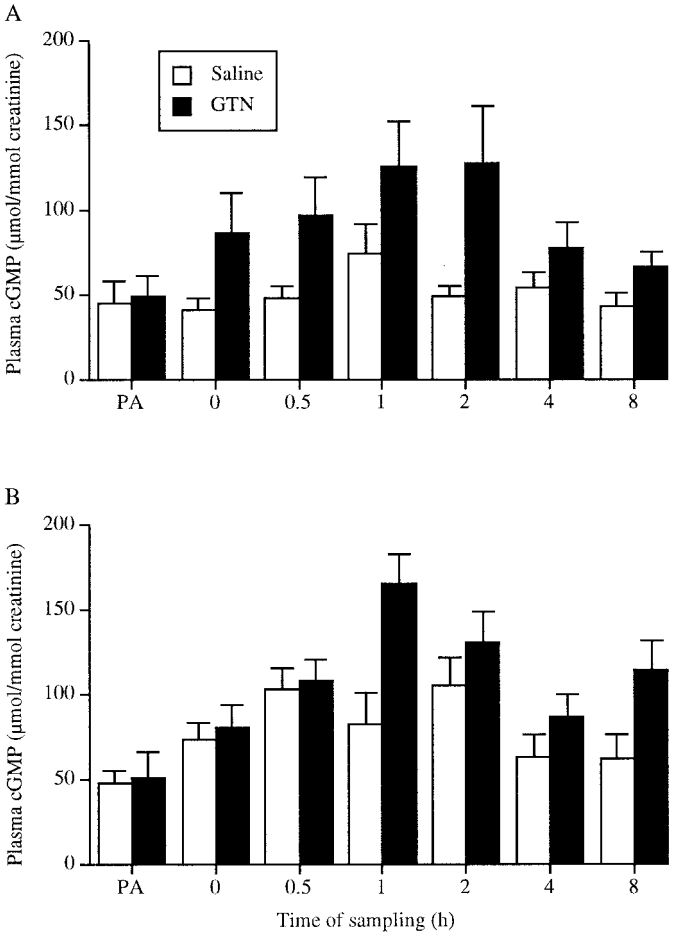

FIG. 3. Changes in plasma cGMP before (PA, before anesthesia), during (first 2 h of sampling), and after cardiac surgery in patients with diabetes (*A*) and without diabetes (*B*) receiving an infusion of saline or GTN at a rate of 1 μg · kg^{-1} · min^{-1} starting 10 min before the institution of CPB and then maintained for a period of 4 h after the initiation of CPB. Values were expressed as the arithmetic mean of 10 patients per group, and bars represent the SE.

the latter, and this was matched by commensurate elevations in plasma cGMP levels. Our study has also demonstrated that the administration of GTN significantly reduces plasma NOx and its urinary elimination in patients with diabetes but has no significant effect on plasma cGMP levels. These results provide new information on the metabolism of NO during cardiac surgery in patients with diabetes, and the scientific and the clinical implications of these findings are further discussed below.

NO metabolism during cardiac surgery. The first major finding of our study is that NO production increased during cardiac surgery in patients with diabetes, as indicated by the increased plasma levels of NOx and cGMP. In addition, this study shows that the NOx and cGMP plasma values were not significantly affected in patients without diabetes, a finding that is supported by other investigators (24–26). These results may be explained by the greater NO production in patients with diabetes under basal conditions (e.g., before surgery) seen in the present study and also reported by other investigators (5). However, it is unlikely that the findings are attributable to changes in renal function, because this was unaffected in both groups of patients. Sharma et al. (25) have shown that during cardiac surgery, NO is not elevated in the coronary effluent of patients with diabetes, suggesting that the source of the increase in NO production seen in our study is not the heart.

The reason for the greater NO production in patients with diabetes is unclear, but an increase in the expression of NOS in these patients may play a role. High glucose levels previously shown to increase the expression of constitutive NOS (27) cannot explain the results of the present study because blood glucose values were well controlled at all times (Table 4). It is therefore unlikely that blood glucose levels could have influenced NOS expression or activity in these patients. An alternative explanation for the increase in NO production in the group with diabetes may be the occurrence of hyperinsulinemia. Indeed, there is evidence in the literature for insulin increasing NOS expression (18) and NO production (19–21); however, the observed increase in plasma insulin during cardiac surgery in our study was not followed by an increase in NO in patients without diabetes, and the time-course of the NO elevation seen in patients with diabetes was not related to that of insulin (Figs. 1 and 5). In addition, the infusion of GTN, which blunted NO production in the group with diabetes, delayed but did not suppress the elevation of plasma insulin, which further

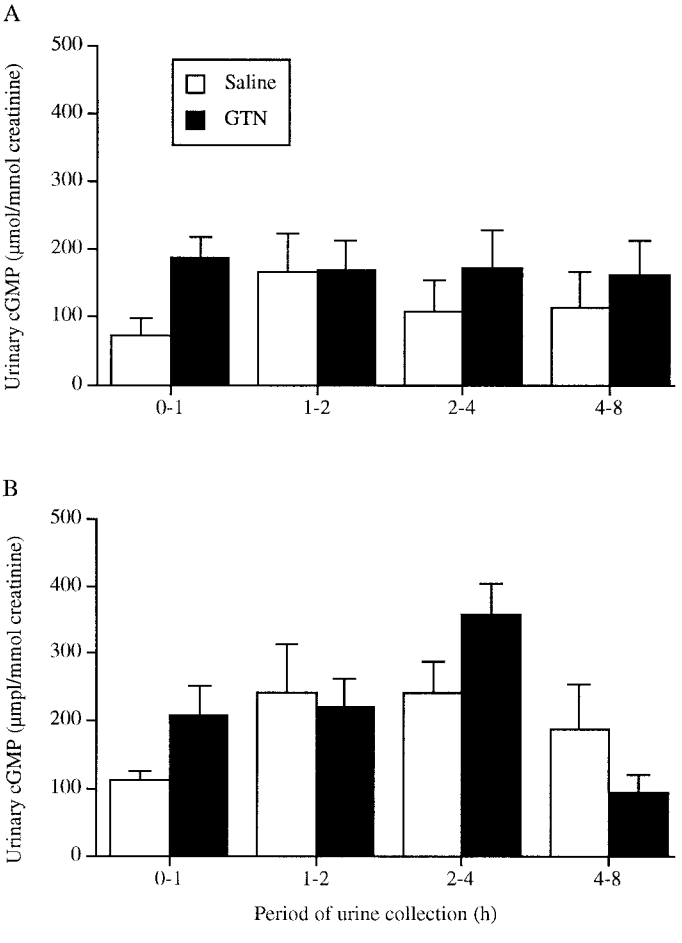

FIG. 4. Changes in urinary cGMP excretion during (first 2 h of sampling) and after cardiac surgery in patients without diabetes (*A*) and with diabetes (*B*) receiving an infusion of saline or GTN at a rate of 1 μg · kg^{-1} · min^{-1} starting 10 min before the institution of CPB and then maintained for a period of 4 h after the initiation of CPB. Values were expressed as the arithmetic mean of 10 patients per group, and bars represent the SE.

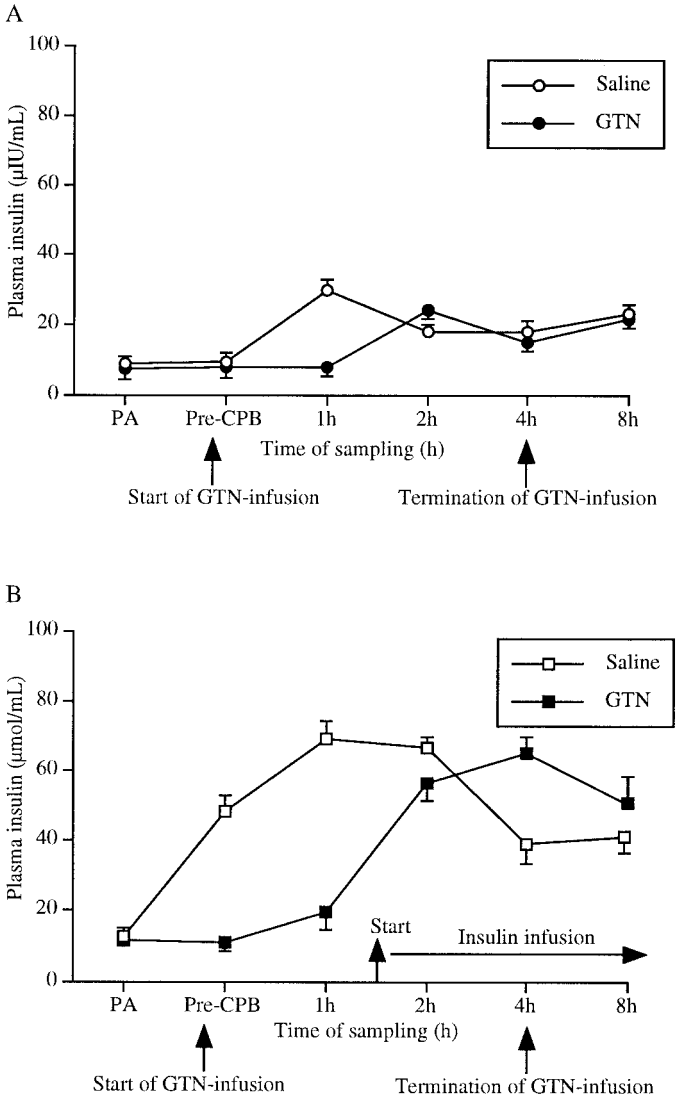

FIG. 5. Changes in plasma insulin before (PA, before anesthesia), before CPB initiation, during (first 2 h of sampling), and after cardiac surgery in patients with diabetes (*A*) and without diabetes (*B*) receiving an infusion of saline or GTN at a rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ starting 10 min before the institution of CPB and then maintained for a period of 4 h after the initiation of CPB. Values were expressed as the arithmetic mean of 10 patients per group, and bars represent the SE.

exposes a lack of association between NO production and hyperinsulinemia during cardiac surgery. It is of interest that the levels of HbA_1 before surgery in patients with diabetes were within the normal range, confirming that the metabolic control in this group was adequate. It is also unlikely that inflammatory factors produced during CPB mediated the increased NO production, because the time-course of factors such as proinflammatory cytokines has no temporal relationship with the time-course of NO (28). The early increase of NO production suggests an involvement of the constitutive NOS rather than the inducible NOS, whose expression and synthesis requires several hours and generates large amounts of NO over long periods of time (7).

The effect of the increase in NO production during CPB on the well being of patients was not under investigation in this study; however, this is still the subject of controversy in the literature. Several investigators have shown that NO may be protective to the ischemic myocardium (29–31), whereas others (32–36) have reported the contrary. In recent studies, our laboratory has demonstrated that NO had no detrimental effects on the human myocardium during ischemia/reperfusion (37), but other studies have shown that the interaction of NO with other free radical species promotes oxidative stress (32,33), nitration of proteins (34,35), and modification of enzyme activity (36) that can be detrimental. As for NO, the consequences of increases in cGMP also remain unresolved. Evidence from experimental heart transplantation (29) has suggested that elevated cGMP improves myocardial preservation and graft blood flow. In contrast, other investigators have reported that activation of the cGMP-dependent protein kinase G pathway can induce DNA fragmentation and apoptotic death of cardiac myocytes (33). It is clear that further work is required to define the role of NO metabolism for patients with diabetes-associated cardiovascular diseases.

Regulation of NO metabolism by GTN. The second major finding of this study is that GTN influences NO metabolism during cardiac surgery. Thus, infusion of GTN significantly reduced plasma NOx levels in patients with diabetes. Our study has not revealed the mechanism of this GTN effect; however, it is possible that GTN down-regulates constitutive NOS activity, a thesis that finds support in the observation that NO acts as a negative feedback modulator of all three NOS isoforms (38–40). Another possible mechanism is GTN reducing in the cellular uptake of L-arginine, the precursor of NO. In this connection, there is recent evidence that GTN suppresses the uptake of L-arginine by cultured bovine endothelial cells after 1–4 h of treatment (41). The above two mechanisms are not mutually exclusive and may both contribute to the GTN-induced reduction of plasma NOx in patients with diabetes (diagram in Fig. 6). It is worth noting that although the plasma NOx mean values were not significantly affected by GTN in patients without diabetes, the observed decrease in urinary NOx excretion suggests that GTN also reduces NO production in this group of patients.

The absence of significant changes in plasma cGMP during cardiac surgery in patients without diabetes and the increase in plasma values in patients with diabetes were expected because plasma NOx values were unaffected in

TABLE 4
Perioperative blood glucose

	Saline	GTN	*P*
Without diabetes			
Pre-CPB	5.7 ± 0.2	6.0 ± 0.4	0.99
End of CPB	7.0 ± 0.6	6.8 ± 0.4	0.84
2 h	7.3 ± 0.6	7.4 ± 0.6	1.99
4 h	8.0 ± 0.5	8.8 ± 0.6	0.96
8 h	6.9 ± 0.4	7.8 ± 0.7	0.78
With diabetes			
Pre-CPB	10.9 ± 1.7*	9.4 ± 0.8*	0.62
End of CPB	10.0 ± 0.7*	10.6 ± 0.5*	1.02
2 h	11.0 ± 0.6*	10.8 ± 0.6*	1.23
4 h	10.2 ± 0.4*	10.6 ± 0.4*	0.93
8 h	11.6 ± 0.8*	9.6 ± 0.7*	0.84

Data are means ± SD in millimoles per liter. *$P < 0.05$ versus the corresponding mean values in the group without diabetes.

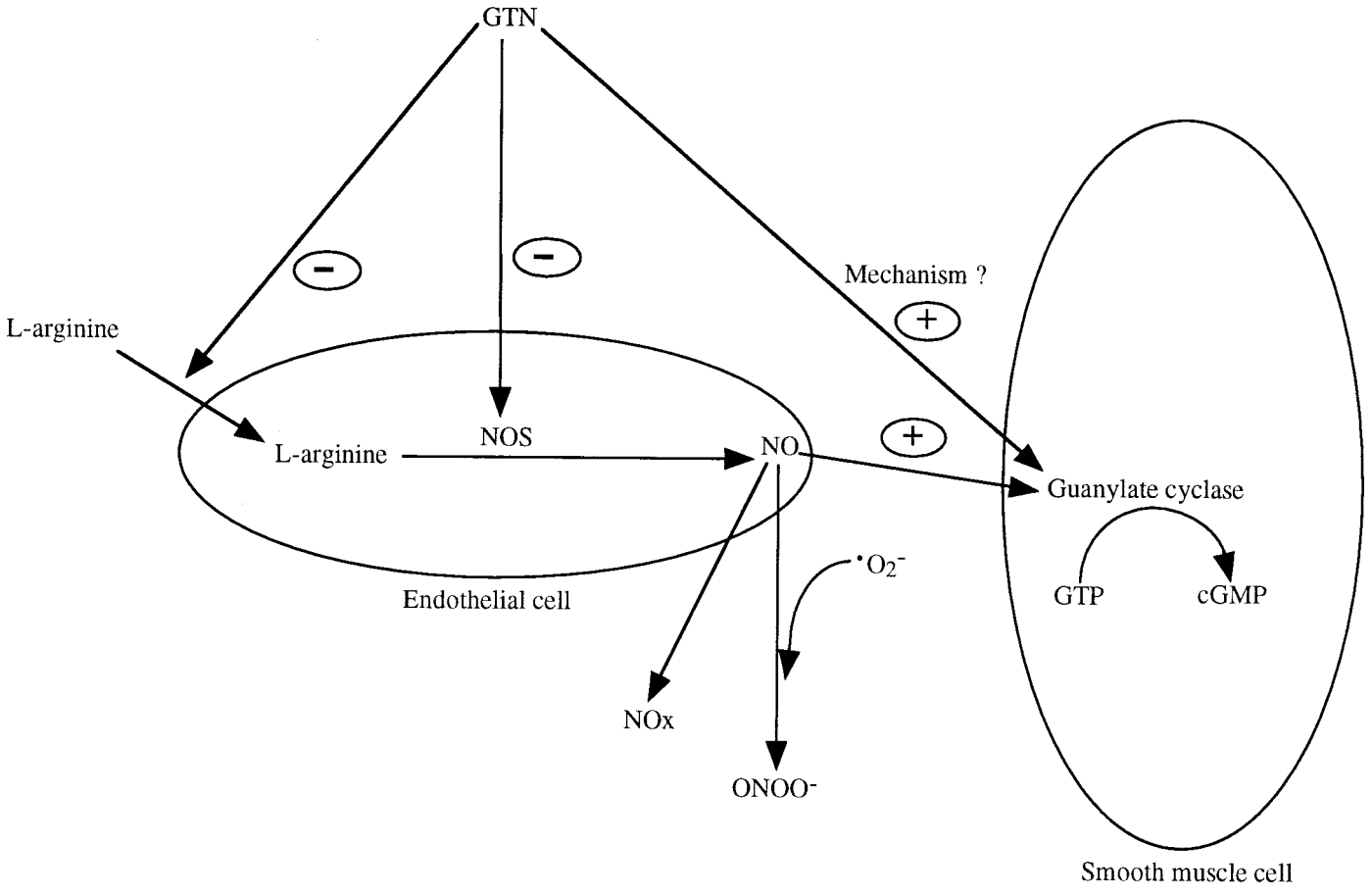

FIG. 6. Proposed mode of action of GTN on NO and cGMP formation. −, Inhibition; +, stimulation.

the former and elevated in the latter. However, the observed increase in plasma cGMP induced by GTN in patients without diabetes, in whom plasma NOx was unaffected by GTN, and the consistent elevation of plasma cGMP in patients with diabetes, in whom GTN decreased plasma NOx, is at first sight difficult to interpret. This dissociation between plasma NOx and cGMP caused by the administration of GTN in both groups of patients may find an explanation in the kinetics and metabolism of GTN. The mechanism by which GTN affects NO metabolism remains undefined (42,43), but it is possible to speculate that GTN acts directly or indirectly on the enzyme guanylate cyclase by a mechanism that is NO-independent (Fig. 6). A recent study using isolated rabbit aorta and electron paramagnetic resonance with an NO spin-trapping agent has shown that NO is not generated from GTN (44), further suggesting that the action of GTN on guanylate cyclase and the generation of cGMP are not mediated by NO. A recent report by Piatti et al. (45) has shown that in insulin-resistant nondiabetic subjects, plasma NO is elevated, whereas cGMP is reduced. This report also lends support to the thesis that under certain conditions cGMP may be regulated by an NO-independent mechanism.

The mechanism by which GTN reduces cGMP clearance in patients with and without diabetes and NOx clearance in those with diabetes is unknown, but it is possible that some renal mechanism is central to the effects. The reduced clearance of cGMP resulted in a significant increase in plasma cGMP and, as discussed above, this may have important consequences, such as modulation of the vascular tone and blood flow of tissues, including the kidneys.

The effect of GTN on the regulation of NO and cGMP formation seen during cardiac surgery in this study may have important clinical implications for cardiac surgery and other clinical conditions. It may be particularly relevant in disease states such as diabetes, where basal NO formation is increased. The mechanism(s) of the GTN effects remains unclear, as does the clinical consequences of its actions, and both require further investigation before NO donor agents are considered as a tool for the modulation of NO and cGMP production.

ACKNOWLEDGMENTS

This study was partially supported by grants from the Glenfield Hospital NHS Trust, the Royal College of Surgeons of U.K., and Link-Up and Heart Link Trusts. We would like to thank Dr. Melanie Davies (Department of Endocrinology, Leicester General Hospital) for useful advice on patient selection. We acknowledge Drs. David Duthie and J. Swanevelder (Department of Anesthesia, Glenfield Hospital) for administering anesthesia to the patients involved in this study. We also acknowledge Nick Taub (Department of Epidemiology and Public Health, University of Leicester) for the advice on statistical analyses. Lastly, we acknowledge the contribution of the late Professor David de Bono (Professor of Medicine, Division of Cardiology, University of Leicester) for support during this study.

REFERENCES

1. Kannel WB, McGee DL: Diabetes and cardiovascular risk factors: the Framingham study. *Circulation* 59:8–13, 1979
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 without diabetes mellitus. *Am J Cardiol* 81:7–11, 1998
3. Fietsam RJ, Bassett J, Glover JL: Complications of coronary artery surgery in diabetics. *Am Surg* 96:551–557, 1996
4. Durante W, Sen AK, Sunahara FA: Impairment of endothelium-dependent relaxation in aortae from spontaneously diabetic rats. *Br J Pharmacol* 94:463–468, 1988
5. Catalano M, Garzaniga G, Perilli E, Jun T, Scandale G, Andreoni S, Carotta M: Basal nitric oxide is not reduced in patients with noninsulin-dependent diabetes mellitus. *Vascular Med* 2:302–305, 1997
6. Matata BM, Galiñanes M: Cardiopulmonary bypass exacerbates oxidative stress but does not increase proinflammatory cytokines release in patients with diabetes compared with patients without diabetes: regulatory effects of exogenous nitric oxide. *J Thorac Cardiovasc Surg* 120:1–11, 2000
7. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43:109–142, 1991
8. Murad F: Cyclic guanosine monophosphate as a mediator of vasodilation. *J Clin Invest* 78:1–5, 1986
9. Chin JH, Azhar S, Hoffman BB: Inactivation of endothelial derived relaxing factor by oxidized lipoproteins. *J Clin Invest* 89:10–18, 1992
10. Petros A, Bennett D, Vaillance P: Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* 338:1557–1558, 1991
11. Cuthbertson BH, Galley HF, Webster NR: The effect of nitric oxide and peroxynitrite on interleukin-8 and elastase from lipopolysaccharide-stimulated whole blood. *Anesth Analg* 86:427–431, 1998
12. Radi R, Beckman JS, Bush KM, Freeman BA: Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 288:481–487, 1991
13. Horie Y, Wolf R, Granger DN: Role of nitric oxide in gut ischemia-reperfusion-induced hepatic microvascular dysfunction. *Am J Physiol* 273:G1007–G1013, 1997
14. Ignarro LJ: Biosynthesis and metabolism of endothelium-derived nitric oxide. *Ann Rev Pharmacol Toxicol* 30:535–560, 1990
15. Tolins JP, Palmer RMJ, Moncada S, Raij L: Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. *Am J Physiol* 27:H655–H662, 1990
16. Wennmalm A, Benthin G, Edlund A, Jungersten L, Kieler-Jensen N, Lundin S, Westfelt UN, Petersson A-S, Waagstein F: Metabolism and excretion of nitric oxide in humans: an experimental and clinical study. *Circ Res* 73:1121–1127, 1993
17. Böger RH, Bode-Böger SM, Thiele W, Junker W, Alexander K, Frölich JC: Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. *Circulation* 95:2068–2074, 1997
18. Aljada A, Saadeh R, Assian E, Ghanim H, Dandona P: Insulin inhibits the expression of intercellular adhesion molecule-1 by human aortic endothelial cells through stimulation of nitric oxide. *J Clin Endocrinol Metab* 85:2572–2575, 2000
19. Sinha AK, Bhattacharya S, Acharya K, Mazumder S: Stimulation of nitric oxide synthesis and protective role of insulin in acute thrombosis in vivo. *Life Sci* 65:2687–2696, 1999
20. Tsukahara H, Kikuchi K, Tsumura K, Kimura K, Hata I, Hiraoka M, Sudo M: Experimentally induced acute hyperinsulinemia stimulates endogenous nitric oxide production in humans: detection using urinary NO2-/NO3- excretion. *Metabolism* 46:406–409, 1997
21. Zavaroni I, Platti PM, Monti LD, Gasparini P, Barilli LA, Massironi P, Ardigo D, Valsecchi G, Delsignore R, Reaven GM: Plasma nitric oxide concentrations are elevated in insulin-resistant healthy subjects. *Metabolism* 49:959–961, 2000
22. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
23. Alberti KGMM, Zimmet PZ, for the WHO Consultation: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation *Diabet Med* 15:539–553, 1998
24. Ihnken K, Winkler A, Schlensak C, Sarai K, Neidhart G, Unkelbach U, Mülsch A, Sewell A: Normoxic cardiopulmonary bypass reduces oxidative myocardial damage and nitric oxide during cardiac operations in the adult. *J Thorac Cardiovasc Surg* 116:327–334, 1998
25. Sharma AC, Fogelson BG, Nawas SI, Vigneswaran WT, Sam AD, Alden KJ, Ferguson JL, Law WR: Elevated coronary endothelin-1 but not nitric oxide in diabetics during CABG. *Ann Thorac Surg* 67:1659–1663, 1999
26. Brett SJ, Quinlan GJ, Mitchell J, Pepper JR, Evans T: Production of nitric oxide during surgery involving cardiopulmonary bypass. *Crit Care Med* 26:272–278, 1998
27. 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* 96:25–28, 1997
28. Steinberg JB, Kapelanski DP, Olson JD, Weiler JM: Cytokine and complement levels in patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 106:1008–1016, 1993
29. Pinsky DJ, Oz MC, Koga S, Taha Z, Broekman MJ, Marcus AJ, Liao H, Naka Y, Brett J, Cannon PJ, Nowygrod R, Malinski T, Stern DM: Cardiac preservation is enhanced in a heterotopic rat transplant model by supplementing the nitric oxide pathway. *J Clin Invest* 93:2291–2297, 1994
30. Weyrich AS, Ma X-L, Lefer AM: The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. *Circulation* 86:279–288, 1992
31. Fukuda H, Sawa Y, Kadoba K, Taniguchi K, Shimazaki Y, Matsuda H: Suppl. of nitric oxide attenuates neutrophil-mediated reperfusion injury. *Circulation* 92 (Suppl. II):II413–II416, 1995
32. Kroncke K-D, Fehsel K, Kolb-Bachofen V: Nitric oxide: cytotoxicity versus cytoprotection–how, why, when, and where? *Nitric Oxide* 1:107–120, 1997
33. Shimojo T, Hiroe M, Ishiyama S, Ito H, Nishikawa T, Marumo F: Nitric oxide induces apoptoptic death of cardiomyocytes via a cyclic-GMP-dependent pathway. *Exp Cell Res* 247:38–47, 1999
34. Wang P, Zweier JL: Measurement of nitric oxide and peroxynitrite generation in the postischemic heart: evidence for peroxynitrite-mediated reperfusion injury. *J Biol Chem* 271:29223–29230, 1996
35. Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin JC, Smith CD, Beckman JS: Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch Biochem Biophys* 298:431–437, 1992
36. Cleeter MWJ, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AVH: Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain by nitric oxide: implications for neurodegenerative diseases. *FEBS Lett* 345:50–54, 1994
37. Zhang J-G, Galiñanes M: Role of L-arginine-nitric oxide pathway in ischemic/reoxygenation injury of the human myocardium. *Clin Sci* 99:497–504, 2000
38. Buga GM, Griscavage JM, Ignarro LJ: Negative feedback regulation of endothelial cell function by nitric oxide. *Circ Res* 73:808–812, 1993
39. Griscavage JM, Rogers NE, Sherman MP, Ignarro LJ: Inducible nitric oxide synthase from a rat alveolar macrophage cell line is inhibited by nitric oxide. *J Immunol* 151:6329–6337, 1993
40. Griscavage JM, Fukuto JM, Komori Y, Ignarro LJ: Nitric oxide inhibits neuronal nitric oxide synthase by interacting with the heme prosthetic group: role of tetrahydrobiopterin in modulating the inhibitory action of nitric oxide. *J Biol Chem* 269:21644–21649, 1994
41. Abou-Mohammed G, Kaesemeyer WH, Caldwell RB, Caldwell RW: Role of L-arginine in the vascular actions and development of tolerance to nitroglycerin. *Br J Pharmacol* 130:211–218, 2000
42. Hill KE, Hunt RW Jr, Jones R, Hoover RL, Burk RF: Metabolism of nitroglycerin by smooth muscle cells: involvement of glutathione and glutathione S-transferase. *Biochem Pharmacol* 43:561–566, 1992
43. Kurz MA, Boyer TD, Whalen R, Peterson TE, Harrison DG: Nitroglycerin metabolism in vascular tissue: role of glutathione S-transferases and relationship between NO and NO_2^- formation. *Biochem J* 292:545–550, 1993
44. Kleschyov A, Mollnau H, Meinertz T, Munzel T: No NO from nitroglycerin (Abstract)? *Circulation* 102: II–245, 2000
45. Piatti PM, Monti LD, Zavaroni I, Valsecchi G, Van Phan C, Costa S, Conti M, Sandoli EP, Solerte B, Pozza G, Pontirolli AE, Reaven G: Alterations in nitric oxide/cyclic-CMP pathways in nondiabetic siblings of patients with type 2 diabetes. *J Clin Endocrin Metab* 85:2416–2420, 2000