Glomerular thrombosis in pregnancy: Role of the L-arginine-nitric oxide pathway

LEOPOLDO RAIJ, with the technical assistance of KAREN COFFEE, JANETH GUERRA, and DAWN HOLMES

Department of Medicine, Veterans Affairs Medical Center and University of Minnesota Medical School, Minneapolis, Minnesota, USA

Glomerular thrombosis in pregnancy: Role of the L-arginine-nitric oxide pathway. *E. coli* endotoxin (LPS) and certain cytokines induce synthesis of nitric oxide (NO) from L-arginine, but also promote endothelial injury and intravascular coagulation. NO has vasodilator and antithrombogenic properties. We investigated the relationship between the L-arginine-NO pathway and the susceptibility to LPS-induced glomerular thrombosis in pregnancy. Pregnant rats were given either 0.15 or 0.75 mg/kg/body wt of LPS intraperitoneally. In rats given 0.15 mg/kg/body wt of LPS urinary NO_2^-/NO_3^- (end products of NO) increased 200% ($P < 0.05$), plasma L-arginine did not change, and glomerular thrombosis was minimal. Pregnant rats given 0.75 mg/kg/body wt of LPS developed glomerular thrombosis in 75% of glomeruli ($P < 0.05$). In these rats plasma L-arginine fell 98%, from 53 ± 4 to 1.4 ± 0.9 mmol/liter ($P < 0.05$) but the urinary NO_2^-/NO_3^- did not increase. Oral administration of L-arginine but not D-arginine increased urinary NO_2^-/NO_3^- by 250% and averted glomerular thrombosis in these rats ($P < 0.05$). Virgin rats given 0.75 mg/kg/body wt of LPS did not contract glomerular thrombosis. In these rats plasma L-arginine decreased only 40% while urinary NO_2^-/NO_3^- concomitantly increased over 200% ($P < 0.05$). Plasma endothelin-1 increased only in rats exhibiting glomerular thrombosis. Thus, limited maternal reserve capability for NO synthesis may underlie, at least in part, the susceptibility for glomerular thrombosis in pregnancy.

E. coli lipopolysaccharaide (LPS) is an endotoxin which has been associated with the syndrome of septic shock, often accompanied by intravascular coagulation [1, 2]. Alterations in the vascular endothelium, induced directly by LPS and/or substances synthesized in response to LPS, such as interleukin-1, tumor necrosis factor and platelet activating factor [3, 4] are believed to underlie many of the clinical and pathological manifestations of endotoxemia. Animals [5–7] and humans [8–10] are known to be more susceptible in late pregnancy to development of glomerular thrombosis and renal failure due to pathologic processes that result in endothelial injury and increased blood coagulability. In animal species such as the rat, which is resistant to LPS, or the rabbit, in which two consecutive doses of LPS are required for thrombogenesis, glomerular thrombosis readily occurs in response to a single dose of LPS if it is given close to the time of delivery [6, 7]. Similarly, pregnant

humans are vulnerable to development of glomerular thrombosis as a complication of disease processes accompanied by endothelial injury and increased thrombogenicity. These conditions include preeclampsia, the HELLP syndrome (Hemolysis, Elevated Liver enzymes, and Low Platelet counts), and septic abortion [8, 10, 11]. Decreased synthesis of prostacyclin and increased synthesis of thromboxane, resulting in platelet aggregation and increased sensitivity to vasoconstrictors such as angiotensin II, have been suggested as important contributors to the pathophysiology of these disease processes [8, 10, 12, 13]. Moreover, several studies have shown that plasma endothelin-1, a powerful vasoconstrictor peptide is increased in preeclampsia [14, 15].

Nitric oxide (NO) is an endogenous vasodilator synthesized by NO synthases from L-arginine [16–20]. NO inhibits platelet aggregation and adhesion to subendothelial collagen [21–23]. The platelet antiaggregatory property of NO is synergistic with that of prostacyclin [21]. Two forms of NO synthase have been identified: Type I synthase can be induced by LPS and/or cytokines such as tumor necrosis factor and interleukin-1 in several types of cells, including endothelial cells, macrophages, vascular smooth muscle, and mesangial cells of the renal glomerulus [16, 19, 24–29]. Type II synthase is constitutive; its prototype is that present in the endothelium [16–18, 30, 31]. Protracted synthesis of large amounts of NO by Type I synthase has been associated with the late and often vasopressor-resistant vasodilation of septic shock [32–34]. Increases in serum levels and in urinary excretion of nitrite and nitrate anions (NO_2^-/NO_3^-), the end products of NO, have been found in animals given endotoxin [35], and recently in humans treated with interleukin-2 [28].

We have shown that the rat responds to a single dose of LPS with a two- to threefold increase in serum and in urinary NO_2^-/NO_3^- [35]. These rats also had a concomitant increase in urinary 3'-5' cyclic guanosine monophospate (cGMP), the end product of guanylate cyclase activation by NO [16, 18, 20]. We identified the glomeruli as the major source within the kidney of the NO response to LPS [35]. Glomerular mesangial cells are likely major contributors, since we and others have shown in these cells induction of NO synthesis in response to LPS as well as to several cytokines [27, 29]. We have also demonstrated that administration of L-nitroarginine methylester (L-NAME), an inhibitor of NO synthesis [16], to these rats prevents the urinary increase of NO_2^-/NO_3^- and cGMP and concomitantly leads to

Table 1. Urinary NO_2^-/NO_3^- excretion and plasma endothelin in pregnant rates

Groups	LPS dose mg/kg/body wt	Urine NO_2^-/NO_3^- nmol $NO_2^-/NO_3^-/\mu g$ creatinine	Plasma ET fmol/ml
I	Control	1.2 ± 0.1 (11)	13.3 ± 1.1 (9)
II	0.15	2.5 ± 0.3 (5)[a]	12.4 ± 0.6 (6)
III	0.75	1.09 ± 0.1 (6)	65 ± 3.4 (5)[c]
IV	0.75 + 2% L-arg	3.0 ± 0.8 (7)[a]	16 ± 1.3 (7)
V	0.75 + 2% D-arg	1.4 ± 0.1 (6)	34.1 ± 5.6 (6)[c]
VI	0.15 + L-NAME	0.5 ± 0.1 (6)	37.1 ± 3.0 (6)[c]
VII	L-NAME	0.5 ± 0.1 (5)	11.1 ± 0.6 (6)

Number of rats is in parentheses. Urinary excretion of NO_2^-/NO_3^- and plasma endothelin-1 (ET), in 20-day pregnant control and experimental rats given intraperitoneal saline or LPS, in a dose of either 0.15 mg/kg body wt or 0.75 mg/kg body wt. Group IV recieved 2% L-arginine (L-arg), group V 2% D-arginine, (D-arg) and groups VI and VII L-nitroarginine methylester (L-NAME) (25 mg/dl) in the drinking water from day 13th to 20th of pregnancy.

[a] $P < 0.05$ vs. control
[b] $P < 0.05$ vs. control; 0.15 LPS + L-NAME; L-NAME
[c] $P < 0.05$ vs. control; 0.75 LPS + 2% L-ARG; L-NAME

generalized glomerular thrombosis in response to a single dose of LPS [35]. We therefore concluded that in endotoxemia, while excessive NO synthesis may result in unwanted severe hypotension, a critical amount of NO is necessary to maintain renal perfusion, maintain medullary oxygentation [36] and prevent platelet aggregation and thrombosis, which are common complications of this form of endothelial injury [6, 35].

Plasma levels of certain aminoacids including L-arginine, the unique substrate for NO synthesis, are decreased in animals and humans in late pregnancy [37–39]. However, amino acid levels in cord blood are either normal or high [37, 38]. Several studies have shown that maternal hypoaminoacidemia, including hypoargininemia, is in part due to active transport of amino acids to the fetus [37, 38]. Whether or not an absolute or relative insufficiency in maternal synthesis of aminoacids is also contributory is currently unknown. Prompted by this fact and by our previous findings suggesting a renal protective role of NO in endotoxemia [35] we investigated in pregnant rats: (a) whether there is a relationship between LPS induced NO synthesis, plasma L-arginine levels and susceptibility to glomerular thrombosis; and (b) whether there is a relationship between glomerular thrombosis, NO synthesis and endothelin-1 (ET), the powerful vasoconstrictor of endothelial origin [40].

Methods

Thirteen day pregnant rats and age-matched female virgin rats were obtained from Harlan Laboratories (Indianapolis, Indiana, USA). The rats were maintained in a germ free environment, fed standard rat chow (Purina, St. Louis, Missouri, USA) and had free access to tap water or water containing either 2% L-arginine hydrochloride (L-arg), 2% D-arginine hydrochloride (D-arg) or 25 mg/dl of the inhibitor of NO synthesis, L-NAME. On the 20th day of pregnancy, after an overnight fast, but with access to water, groups of rats (Table 1) were given either 1 cc/kg body wt of sterile normal saline (NS) or NS containing E. coli lipopolysaccharide (LPS) serotype 0127: D8 (Difco Laboratories, Detroit, Michigan, USA) in-

traperitoneally (i.p.) according to the experimental protocols described below (Table 1).

Pregnant rats

Group I ($N = 11$) were control rats which received only NS i.p.; Group II rats ($N = 6$) received 0.15 mg/kg body wt of LPS in NS; Group III ($N = 6$) received 0.75 mg/kg body wt of LPS in NS; Group IV ($N = 8$) were given 2% L-arg in the drinking water from the 13th to 20th days of pregnancy, and received 0.75 mg/kg body wt LPS i.p.; Group V ($N = 7$) were given 2% D-arg in the drinking water from the 13th to 20th days of pregnancy, and received 0.75 mg/kg body wt LPS i.p.; Group VI ($N = 9$) were given 25 mg/dl of L-NAME in the drinking water from the 13th to 20th days of pregnancy, and received 0.15 mg/kg LPS i.p.; Group VII ($N = 6$) were given 25 mg/dl of L-NAME in the drinking water from the 13th to 20th days and received NS i.p.

Virgin rats

These rats were maintained in the same environment and fed the same diet as pregnant rats and had free access to water. Six virgin rats received NS i.p. and six received LPS, 0.75 mg/kg/body wt i.p.

After administration of LPS or NS, rats were placed in individual metabolic cages, and timed urine collections were obtained over four to six hours for measurement of creatinine and NO_2^-/NO_3^-. The rats were then anesthetized with Inactin (BYK Gulden Konstanz, Germany; 100 mg/kg i.p.), the abdominal aorta was exposed through a midline incision, and the rats killed by exsanguination. The blood obtained was used for determination of plasma L-arg. A few urine and/or blood samples from some rats could not be used due to technical problems such as spilling, contamination or misplacement after collection. The actual number of samples studied is seen in Table 1. Kidney tissue was obtained and prepared for light and immunofluorescence microscopy as previously described [35, 42]. For light microscopy, formalin-fixed, paraffin-embedded 4-μm sections were stained with Masson trichrome. Sections were examined in a blinded manner for evidence of capillary thrombi, and percent glomeruli showing thrombi was quantitated [35, 41]. At least 50 glomeruli were examined in each kidney. In selected tissues, frozen sections were treated with FITC-labeled goat anti-rat polyclonal antibody against fibrinogen (Cappel Laboratories, Durham, North Carolina, USA) to confirm the nature of the thrombi observed [35]. Plasma L-arg ($\mu mol/liter$) was measured with a Beckman 6300 Analyzer (Beckman Instruments, Fullerton, California, USA) in four rats in each experimental group (except groups V and VII) as well as in four age-matched female virgin rats that received NS i.p. and four that received LPS, 0.75 mg/kg/body wt i.p. [42]. For NO_2^-/NO_3^- measurements, samples were first incubated with E. coli reductase to convert NO_3^- to NO_2^-, and then total NO_2^- was measured using the Griess reagent according with methods previously described [27, 35, 43]. Urine creatinine was measured with a Creatinine Analyzer 2 (Beckman). Urine NO_2^-/NO_3^- was expressed as urinary NO_2^-/NO_3^- excretion (nmol/hr/100 g body wt) divided by the urinary creatinine excretion ($\mu g/hr/100$ g body wt). Plasma ET in pregnant rats was measured by RIA (Amersham Corp.) and expressed in fmol/ml [44]. Data are expressed as mean ± SEM; statistical
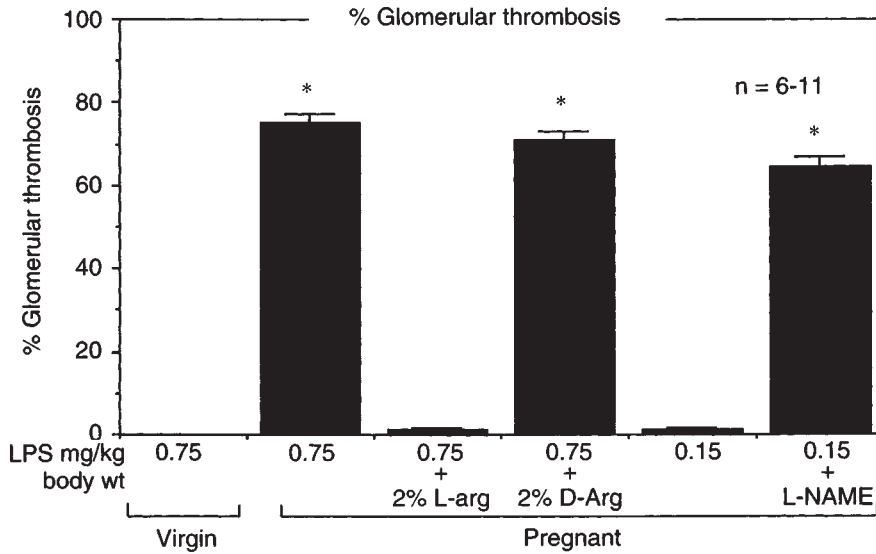
Fig. 1. *Percent glomerular thrombosis in virgin and pregnant rats in the different experimental groups.* Control rats received NS i.p. instead of LPS i.p. 2% L-arginine; 2% D-arginine or L-NAME (25 mg/dl) was given in the drinking water from the 13th to 20th day of pregnancy. *$P < 0.05$ vs. other groups.
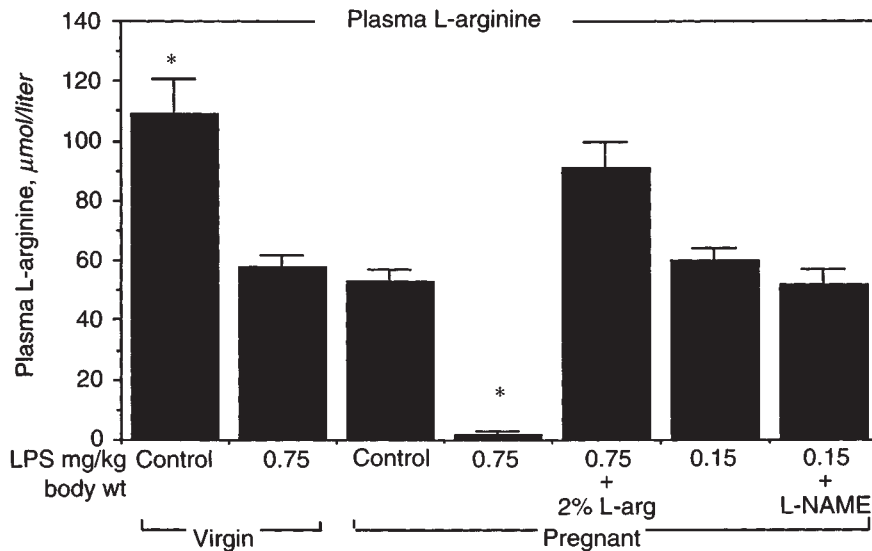

Fig. 2. *Plasma levels of L-arginine (L-arg) in virgin Sprague-Dawley female rats age-matched with pregnant rats in the different experimental groups* (N = 4 in each group). Rats had free access to either tap water or water containing 2% L-arginine (L-arg) or L-nitroarginine methylester (L-NAME) 25 mg/dl from day 13th of pregnancy; on the 20th day, the rats were given *E. coli* lipopolysaccharide (LPS) intraperitoneally in a dose of either 0.15 or 0.75 mg/kg body wt in saline. Plasma L-arg was measured in blood obtained at the time of sacrifice, 4 to 6 hours after administration of either saline or LPS. *$P < 0.05$ vs. 0.75 virgin; control pregnant.

significance of data was evaluated by unpaired Student t-test for comparisons between groups of virgin rats, and by ANOVA for the remaining comparisons (STATVIEW 512 software, Brainpower, Calabases, California, USA); $P < 0.05$ was considered significant.

Results

The basal urinary excretion of the end products of NO, NO_2^-/NO_3^- was 70% higher in pregnant compared to virgin rats (1.2 ± 0.1 vs. 0.70 ± 0.04 mmol NO_2^- /NO_3^-/mg creatinine; $P < 0.05$) suggesting increased NO synthesis in pregnancy [45]. Female virgin rats given 0.75 mg/kg/body wt i.p. of LPS did not develop glomerular thrombosis (Fig. 1) and had a threefold increase in urinary NO_2^-/NO_3^- from 0.70 ± 0.04 to 2.2 ± 0.7 mmol NO_2^-/NO_3^-/μg creatinine ($P < 0.05$) The urinary NO_2^-/NO_3^- excretion as well as the renal histology were markedly different between the groups of pregnant rats which received the two different doses of LPS. The rats given

0.15 mg/kg body wt LPS (Group II) responded in a fashion similar to that in virgin rats, that is, these rats had a significant increase in urinary NO_2^-/NO_3^- of approximately 200% ($P < 0.05$), and negligible glomerular thrombosis (Table 1, Fig. 1). On the other hand, rats given 0.75 mg/kg body wt of LPS (Group III) showed diffuse glomerular thrombosis involving 75% of glomeruli ($P < 0.05$) and had no increase in urinary excretion of NO_2^-/NO_3^- (Table 1, Fig. 1). In addition tubular epithelial cell swelling and desquamation was also noted. Immunofluoresence microscopy confirmed that the material deposited within the glomerular capillary loops was antigenically related to fibrinogen [35].

In agreement with previous studies [37–39] we found that plasma L-arg is depressed by approximately 40% in rats 20 days pregnant compared with age-matched female virgin rats ($P < 0.05$) (Fig. 2). In pregnant rats receiving 0.15 mg/kg body wt of LPS, plasma L-arg levels did not change and remained similar to the levels in control rats (Group I) which received NS i.p.

However, the plasma levels of L-arg decreased by 98% (from 53 ± 4 to 1.4 ± 0.9 μmol/liter) in pregnant rats given 0.75 mg/kg body wt of LPS ($P < 0.05$) while the same dose of LPS resulted in a decrease of only 40% in virgin rats (Fig. 2). In the group of rats which received 2% of L-arg in the drinking water from the 13th day of pregnancy until the 20th, (Group IV) plasma L-arg levels after administration of 0.75 mg/kg body wt of LPS were similar to those in virgin rats (Fig. 2). Likewise, in these rats the increase in urinary NO_2^-/NO_3^- was similar to that observed in virgin rats as well as in rats given 0.15 mg/kg body wt of LPS (Table 1). This represented an increase of 200% in NO_2^-/NO_3^- excretion compared with pregnant control rats and also with rats given 0.75 mg/kg body wt of LPS but no L-arg ($P < 0.05$ vs. both groups). Most important, L-arg supplementation completely prevented glomerular thrombosis ($P < 0.05$) (Fig. 1 and 3). This effect of L-arg was specific, since similar supplementation of rats with L-arg's enantiomer, D-arg, (Group V) neither increased urinary NO_2^-/NO_3^- nor prevented glomerular thrombosis (Table 1, Fig. 1). Administration of the NO synthesis inhibitor L-NAME in the drinking water for 7 days (group VI) inhibited the fall in plasma L-arg (Fig. 2) as well as the increase in urinary NO_2^-/NO_3^- in rats given 0.15 μg/kg body wt of LPS and resulted in the development of glomerular thrombosis in 65% of glomeruli ($P < 0.05$) (Table 1, Fig. 1). On the other hand rats given L-NAME but not LPS (group VII) did not develop glomerular thrombosis.

We found that plasma ET was significantly increased only in the groups of pregnant rats which developed diffuse glomerular thrombosis. Indeed, plasma ET was increased in the rats receiving 0.75 mg/kg body wt but not in those receiving 0.15 mg/kg body wt (Table 1). Similar to what we observed with glomerular thrombosis, L-arg but not D-arg supplementation was effective in arresting the increase in plasma ET in the group of pregnant rats which received 0.75 mg/kg body wt of LPS (Table 1). Furthermore, plasma ET was high in the rats given L-NAME plus LPS but not in those receiving only L-NAME (Table 1).

Discussion

The reasons for the increased susceptibility to glomerular thrombosis in pregnancy have puzzled scientists and clinicians for over 50 years [5, 8].

We obtained rats on the 13th day of pregnancy to test the hypothesis that administration of *Escherichia coli* LPS prepartum, on the 20th day of pregnancy would result in inadequate NO synthesis and therefore in glomerular thrombosis [35]. In agreement with previous studies in humans and in rats we found that plasma L-arg is depressed by approximately 40% in rats 20 days pregnant compared with age-matched female virgin rats [37–39] (Fig. 2). In addition, urinary excretion of NO_2^-/NO_3^- was increased by 70% in pregnant rats. This suggests that increased NO synthesis may mediate, at least in part, the physiologic vasodilation of pregnancy [8, 45]. Urinary NO_2^-/NO_3^- excretion, as well as renal histology, were different between the groups of rats that received either 0.15 or 0.75 mg/kg body wt LPS (Table 1, Fig. 1). Pregnant rats given 0.15 mg/kg body wt LPS responded similarly to female virgin rats given 0.75 mg/kg body wt of LPS, in that they had a significant increase in urinary NO_2^-/NO_3^- and minimal glomerular thrombosis (Tables 1, Fig. 1) [6, 7, 33]. On the other hand, pregnant

rats given 0.75 mg/kg body wt of LPS showed no increase in urinary excretion of NO_2^-/NO_3^- and developed glomerular thrombosis in 75% of glomeruli (Table 1, Fig. 1).

The differences in urinary NO_2^-/NO_3^- between these groups could not be attributed to differences in renal function, because the urinary NO_2^-/NO_3^- was expressed as the ratio between NO_2^-/NO_3^- and urinary creatinine excretion.

We also demonstrated that in those rats receiving 0.15 mg/kg body wt of LPS, plasma L-arg levels did not change and remained similar to the levels in pregnant control rats (Fig. 2). Administration of 0.75 mg/kg body wt to pregnant rats was followed by a 98% fall in plasma levels of L-arg. In contrast, this same dose of LPS resulted in only a 40% fall in plasma L-arg in virgin rats resulting in plasma L-arg levels similar to those observed in pregnant control rats, prior to LPS administration (Fig. 2). Taken together these findings suggested a pathophysiologic link between hypoargininemia, reduced NO synthesis and glomerular thrombosis in pregnant rats given the higher dose of LPS. We therefore studied a group of rats receiving 2% L-arg in their drinking water from the 13th day of pregnancy until the 20th, at which time 0.75 mg/kg body wt LPS was administered. In these rats, plasma L-arg levels did not decrease after LPS administration (Fig. 1). Moreover, in L-arginine supplemented rats, urinary NO_2^-/NO_3^- increased 200% compared with both pregnant-control rats given NS, and with rats given 0.75 mg/kg body wt of LPS but no L-arg in the drinking water. Of particular importance was the finding that L-arg supplementation prevented glomerular thrombosis (Fig. 1). These effects of L-arg were specific, since administration of the L-arginine's enantiomer, D-arg, neither increased urinary NO_2^-/NO_3^- nor prevented glomerular thrombosis (Table 1, Fig. 1).

To further unravel the relationship between NO and glomerular thrombosis in pregnancy, we studied a group of rats given L-NAME [16, 35] in drinking water from the 13th until the 20th days of pregnancy, when 0.15 mg/kg body wt, the nonthrombogenic dose of LPS, was administered. L-NAME, a substituted L-arg derivative, inhibits NO synthesis by competing with L-arg for the NO synthase. In these rats, plasma L-arg did not decrease, urinary NO_2^-/NO_3^- did not increase and, similar to rats that received 0.75 mg/kg body wt of LPS, 65% of glomeruli developed thrombosis (Table 1, Figs. 1 and 2) Comparison of the levels of L-arg between rats that received 0.75 mg/kg body wt of LPS and those that received 0.15 mg/kg body wt plus L-NAME suggests that the susceptibility to glomerular thrombosis in pregnant but not in virgin rats is unlikely to be due to an endogenous inhibitor of NO synthesis in pregnancy. Indeed, in the presence of an inhibitor it would be expected that plasma L-arg would not fall after 0.75 mg/kg body wt similar to what was observed in rats given LPS plus L-NAME (Fig. 2). Urinary NO_2^-/NO_3^- in rats given the inhibitor of NO synthesis L-NAME but not LPS was similar to that in the rats given both L-NAME and LPS. However, glomerular thrombosis was only observed in rats which received both L-NAME and LPS. This suggests that inhibition of NO synthesis by itself does not lead to glomerular thrombosis but predisposes to its development when other factors capable of inducing endothelial injury and increased blood coagulability, such as LPS administration, are also involved [3, 11, 35].

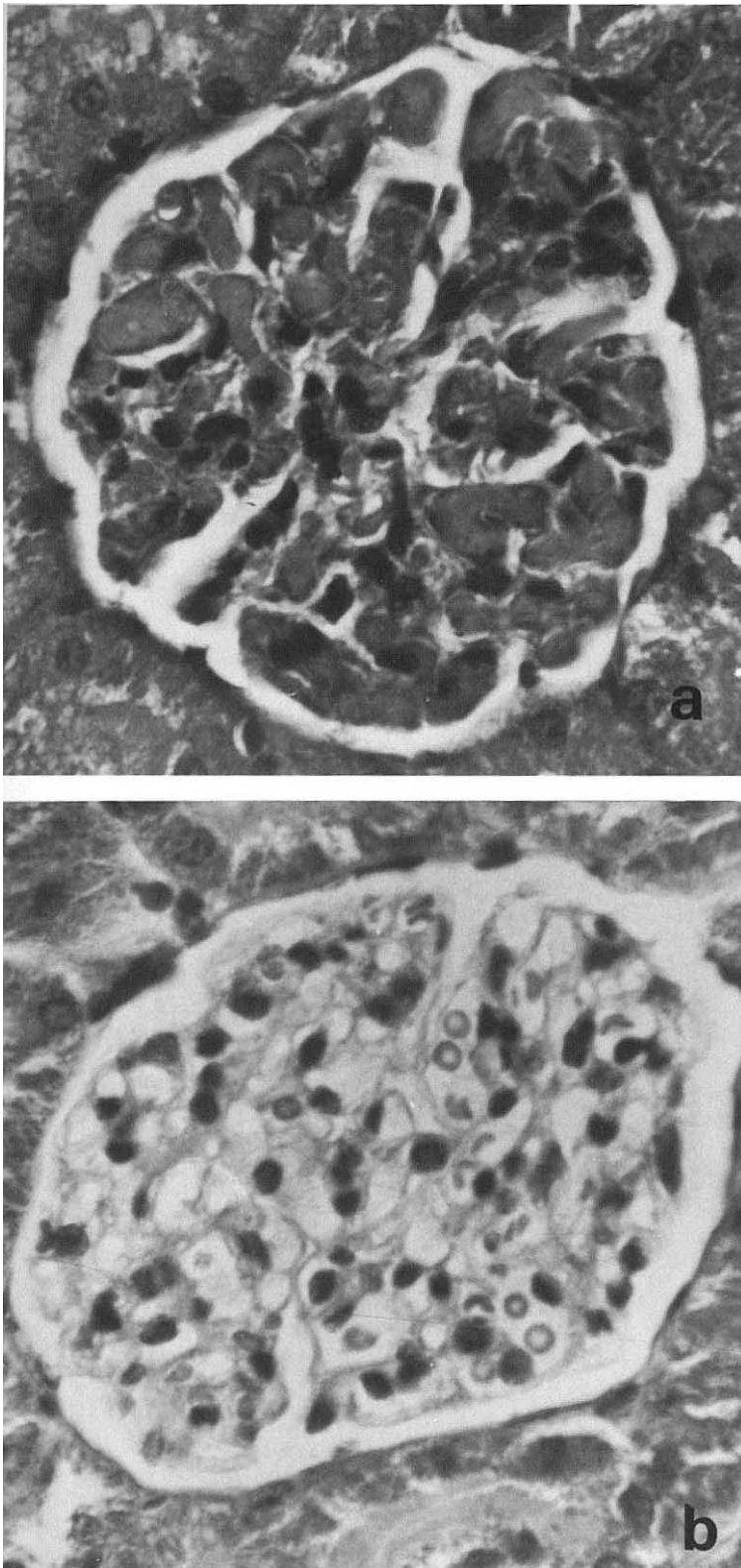The renal tubules are the principal source of plasma L-arg

Fig. 3. *Representative photomicrographs of glomeruli from a 20-day pregnant rat given 0.75 mg/kg body wt LPS intraperitoneally showing thrombi within the capillary loops (a) and a rat given 2% L-arginine in the drinking water from the 13th day of pregnancy and given 0.75 mg/kg body wt LPS on day 20 of pregnancy, showing a normal glomerulus (b). Masson Trichrome, (original magnification × 460).*

[46]. Given the architecture of the renal circulation, in which the renal tubules receive postglomerular blood, L-arg of renal origin would reach the glomerulus once it has circulated sys-temically [47]. Therefore, if there is increased demand for L-arg for synthesis of NO, glomeruli would not enjoy a priority in their supply, which in fact might decrease due to shunting of

L-arg to other tissues. In pregnancy, fetal needs for L-arg take precedence over maternal need, contributing to maternal hypoargininemia [37, 38]. Our results suggest that under conditions of a sudden and robust demand for L-arg to increase NO synthesis, such as that provoked by the larger LPS dose, the fetal needs for L-arg as well as for the extrarenal synthesis of NO [16, 19, 35, 48], may be maintained at the expense of a great diminution in plasma L-arg levels. Moreover, decreased synthesis by injured tubules and increased L-arg catabolism due to increased tissue arginase may establish a vicious circle and contribute to the observed hypoargininemia in response to LPS [49].

Low plasma L-arg would result in insufficient substrate for the synthesis of glomerular NO in amounts adequate to maintain perfusion and prevent thrombosis when there is concomitant endothelial injury [35]. Indeed, L-arginine availability has been shown to be rate-limiting for the synthesis of NO by LPS induced NO synthase in macrophages. The Km of inducible NO synthase in macrophages has been reported to be between 70 and 140 μM [25, 49]. The plasma L-arg levels of pregnant rats given 0.75 mg of LPS/kg body wt fell to 1.4 $\pm$ 0.9 μM. This suggests that low plasma L-arg may, in fact, have played an important role in limiting NO synthesis. This hypothesis is supported by the dramatic and specific effect of L-arg supplementation which increased urinary NO_2^-/NO_3^- and prevented glomerular thrombosis. The salutary affect of increased NO synthesis may be related to both its renal vasodilatory action [20] as well as to its property to inhibit platelet aggregation and adhesion [22, 23].

Endothelial cells synthesize ET in response to a variety of stimuli, including certain forms of endothelial injury, cytokines, and thrombin [48–50]. Studies *in vitro* have shown that inhibition of NO synthesis enhances endothelial ET synthesis induced by thrombin [51]. The kidney is more sensitive to the vasoconstrictor effect of ET than other vascular territories such as the coronary and mesentery arteries [40]. Mesangial cells are also capable of synthesizing ET [40]. Plasma ET is increased in preeclampsia and, transitorily, in some forms of experimental gram-negative endotoxemia [14, 15, 40]. Thus, we considered the possibility that ET may also play a role directly or indirectly in the susceptibility to glomerular thrombosis during pregnancy.

We found that plasma ET was significantly increased only in those pregnant rats with significant glomerular thrombosis. Indeed, plasma ET was only increased in the rats receiving 0.75 mg/kg body wt and in rats given L-NAME plus LPS but not in those receiving only L-NAME. Finally, L-arg but not D-arg supplementation was effective in arresting the increase in plasma ET in the rats receiving 0.75 mg/kg body wt of LPS (Table 1).

These results suggest that neither LPS by itself nor inhibition of NO synthesis alone induces changes in plasma ET in pregnant rats. Rather, in pregnancy, NO may be important in modulating the rise in plasma ET in response to intravascular generation of thrombin and/or to endothelial or glomerular injury [51–53]. Under these circumstances the increase in ET would further decrease glomerular blood flow due to vasoconstriction [40]. In conditions such as preeclampsia and related syndromes, the situation may be further aggravated by the concomitant decreased synthesis of prostacyclin and increased synthesis of thromboxane [8, 10, 11].

In summary, our study suggests for the first time that in late pregnancy, maternal reserve capability to increase NO synthesis may be limited. Hence, unbalanced renal vasoconstriction and thrombosis may result during those disease processes in which Type I NO synthase is induced resulting in a large increase in the synthesis of this endogenous vasodilator and antithrombogenic agent.

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