0031-3998/02/5104-0485
PEDIATRIC RESEARCH
Vol. 51, No. 4, 2002
Printed in U.S.A.

Vasodilation to Vascular Endothelial Growth Factor in the Uterine Artery of the Pregnant Rat Is Blunted by Low Dietary Protein Intake

SHIGERU ITOH, LEE BRAWLEY, TIMOTHY WHEELER, FREDERICK W. ANTHONY, LUCILLA POSTON, AND MARK A. HANSON

Centre for Fetal Origins of Adult Disease, Princess Anne Hospital, University of Southampton, Southampton SO16 5YA, U.K. [S.I., L.B., T.W., F.W.A., M.A.H.], and Department of Obstetrics and Gynecology and Centre for Cardiovascular Biology and Medicine, Guy's King's and St Thomas' School of Medicine, London SE1 7EH, U.K. [L.P.]

ABSTRACT

Pregnancy is associated with a substantial increase in uterine artery blood flow, which may in part result from dilation in response to vascular endothelial growth factor (VEGF). Uterine blood flow is reported to be reduced in globally diet-restricted pregnant rats. Both global and protein dietary restriction in pregnancy produce programmed effects in offspring. In this study we hypothesized that protein restriction in pregnancy impairs maternal uterine artery responses to VEGF. Vascular responses to VEGF were determined in isolated uterine arteries of pregnant (18 or 19 d of gestation) Wistar rats fed a diet containing either 18% or 9% casein throughout pregnancy. For comparison, responses to phenylephrine, potassium chloride, and acetylcholine were determined. In addition, the response of the mesenteric artery to VEGF was studied in the same animals. A significant reduction of the maximal relaxation to VEGF ($p = 0.041$) and in the overall response ($p = 0.004$) to VEGF was found in uterine arteries of the 9% compared with the 18% group, but responses to all other agonists were similar. The VEGF response was reduced by cyclooxygenase inhibition (indomethacin) in both groups. In the 18%, but not the 9%, group it was further reduced by nitric oxide synthase inhibition (*N*ω-nitro-L-arginine methyl ester). VEGF was shown to dilate the mesenteric artery but this effect was not significantly altered by the low-protein diet. These results show an attenuated uterine artery vasodilator response to VEGF produced by a low-protein diet in pregnancy, partly because of a reduction of the nitric oxide component of VEGF-mediated relaxation. **(*Pediatr Res* 51: 485–491, 2002)**

Abbreviations

VEGF, vascular endothelial growth factor
NO, nitric oxide
PGI_2, prostacyclin
PE, phenylephrine
ACh, acetylcholine
L-NAME, *N*ω-nitro-L-arginine methyl ester
INDO, indomethacin
PSS, physiologic salt solution
IC_{100}, internal vessel circumference equivalent to a transmural pressure of 100 mm Hg
KPSS, potassium PSS
pEC_{80}, log PE concentration required to produce 80% of the maximal response to KPSS
pEC_{50}, log of molar concentration producing 50% of the maximal response

Epidemiologic evidence suggests that maternal diet and body composition may be key factors in the links between low birth weight and cardiovascular disease, hypertension, and insulin-independent diabetes in adult life (1–3). These population studies are strongly supported by animal studies. In the rat, a maternal low-protein diet or global undernutrition in pregnancy is associated with reduced birth weight, and elevated blood pressure or impaired glucose tolerance in adult offspring (4–9). The mechanisms that underlie this effect are not known, but their elucidation will be important for future public health measures aimed at improving the nutrition of women of reproductive age.

The provision of nutrients to the fetoplacental unit depends partly on uterine blood flow. Uterine blood flow is increased during normal pregnancy because of vasodilation (10) and vascular remodeling and has been shown to be reduced when pregnant rats are fed a globally restricted diet (11).

VEGF is a specific mitogen (12), which promotes vasodilation and neovascularization, and it has been identified in fetal tissues, the placenta, and also in adult blood vessels (13, 14).

Received June 11, 2001; accepted December 10, 2001.
Correspondence and reprint requests: Mark A. Hanson, M.D., Centre for Fetal Origins of Adult Disease, Level F (887), Princess Anne Hospital, University of Southampton, Coxford Road, Southampton SO16 5YA, U.K.; e-mail: M.Hanson@soton.ac.uk
Supported by The British Heart Foundation and Kanzawa Medical Research Foundation.

Vasodilation in response to VEGF is mediated by the production of NO and PGI_2 through stimulation of endothelial flt-1 and Flk-1/KDR receptors (15). Recently it has been reported in the rat that VEGF mRNA levels increase in the uterine tissue in pregnancy (16). In humans, it has been shown that the maternal plasma concentration of VEGF is increased during pregnancy (17, 18).

The present study was designed to investigate the hypothesis that a maternal low-protein diet affects the uterine circulation during pregnancy through a change in the vasodilator response to VEGF. Experiments were also conducted to determine whether any effect on the response to VEGF was dependent on reduction of NO or PGI_2 production. Preliminary data showed that VEGF-induced NO release is reduced in uterine arteries of the low-protein group (Brawley, Itoh, and Clough, unpublished observation). In the present study we therefore concentrated on examining the effect of inhibition of NO synthase in isolated uterine arteries by removing the prostacyclin component of the VEGF-induced relaxation first using INDO. To determine whether any effects were confined to the uterine artery, the response of the mesenteric artery to VEGF was also studied in the same animals.

METHODS

Animals and dietary protocols. All animal experimentation was conducted in accordance with the U.K. Home Office Animals (Scientific Procedures) Act 1986, and this study was approved by the ethical review process. Twenty-one virgin female Wistar rats, (Harlan, Bicestor, Oxon, U.K.) were studied. Rats were randomly divided into control and low-protein groups. Before pregnancy they were fed standard laboratory chow (CRMX, Special Diets Services, Cambridge, U.K.). Feeding of the synthetic diets began on the day of conception (d 0 of gestation), which was confirmed by observation of semen plugs on the floor of the mating cage. Ten rats in the control group were fed a diet containing 18% casein by weight, and 11 rats were fed a low-protein diet containing 9% casein by weight. All diets contained 5 g/kg methionine (5). In addition, 0.5 g/kg magnesium sulfate was included to prevent magnesium deficiency (Table 1). All rats had free access to food and water and were housed individually in cages in a room with a 12-h light/dark cycle and maintained at 22°C. Animals were killed by CO_2 inhalation on d 18 or 19 of gestation. For each litter, five pups and their placentas were taken at random and weighed after the dissection of vessels.

Table 1. *Composition of the 18% and 9% diets*

Component	18% casein diets	9% casein diets
Casein (g/100 g diet)	18.0	9.0
Sucrose (g/100 g diet)	21.3	24.3
Cellulose fiber (g/100 g diet)	5.0	5.0
Cornstarch (g/100 g diet)	42.5	48.5
Vitamin mix AIN-76 (g/100 g diet)	0.5	0.5
Mineral mix AIN-76 (g/100 g diet)	2.0	2.0
Maize oil (g/100 g diet)	10.0	10.0
Choline chloride (g/100 g diet)	0.2	0.2
Methionine (g/100 g diet)	0.5	0.5
Magnesium sulfate (g/100 g diet)	0.05	0.05

Vitamin mix AIN-76, mineral mix AIN-76, casein, cellulose, and cornstarch were purchased from Special Diet Services.

Determination of vascular function. The uterus and intestine were removed and immediately placed in cold PSS (in mM: NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.17; $NaHCO_3$, 25; KH_2PO_4, 1.18; EDTA, 0.026; and glucose, 5.5). Segments of the main uterine artery (diameter approximately 400 μm) and mesenteric artery (diameter approximately 300 μm) were dissected free from adjoining connective tissue and mounted as a ring preparation on a small vessel wire myograph (Multi Myograph Model 610M, J.P. Trading, Aarhus, Denmark) (19). The arteries were bathed in PSS gassed with a mixture of 95% O_2 and 5% CO_2 (pH 7.4 and 37°C). The passive wall tension–internal circumference relationship was determined by incremental increases in tension, regulated by a microprocessor, to achieve an IC_{100} (using the Laplace relationship), and the arteries were set to a diameter equal to $0.9 \times IC_{100}$. To test viability, vessels were exposed four times to KPSS (125 mM K, equimolar substitution of NaCl with KCl in PSS). Arteries that produced tension equivalent to <100 mm Hg in response to KPSS were excluded from subsequent study.

The following protocol was performed in the uterine artery. Cumulative dose-responses to PE (10^{-8} to 10^{-5} M) and KCl (10^{-3} to 75×10^{-3} M) were conducted. Cumulative relaxation dose-responses to ACh (10^{-8} to 10^{-5} M) and VEGF (10^{-11} to 3×10^{-8} M; human recombinant VEGF, Genentech Inc., San Francisco, CA, U.S.A.) were then assessed after preconstriction with PE. The PE concentration used for preconstriction was that required to produce 80% of the maximal response (pEC_{80}) induced by KPSS. To assess the role of NO synthase, the ACh dose-response curve was repeated after preincubation with the NO synthase inhibitor, L-NAME (10^{-4} M) for 30 min. To assess the role of NO synthase and cyclooxygenase, the VEGF dose-response curve was performed after preincubation with the cyclooxygenase inhibitor INDO (10^{-5} M) and then repeated after preincubation with a combination of INDO and L-NAME for 30 min. In the mesenteric artery, the cumulative dose-response curve to PE (10^{-8} to 3×10^{-5} M) was established, and after preconstriction with PE (pEC_{80}), VEGF dose-response curves (10^{-10} to 10^{-8} M) were studied.

All dose-response curves were conducted with additions of increasing concentration at 2-min intervals, and 20 min was allowed between measurements of each response curve. All drugs and chemicals except VEGF were obtained from Sigma Chemical Co. (Poole, U.K.).

Data analysis. Values are given as mean ± SEM. Tension was expressed as a percentage of maximal constriction to KPSS, to correct for any small differences in vessel diameter. Relaxation was expressed as a percentage of the initial precon-

Table 2. *Maternal and fetal weight*

	18% casein (n = 10)	9% casein (n = 11)
Maternal body weight (g)	304.8 ± 8.4	304.4 ± 7.2
Rate of increase (g/d)	5.28 ± 0.28	5.80 ± 0.71
Number of fetuses	10.8 ± 0.7	11.4 ± 0.6
Mean fetal weight (mg)	2135 ± 231	1960 ± 147
Mean placental weight (mg)	408 ± 16	387 ± 14

Table 3. *Uterine vascular function of control and protein-restricted dams*

	18% casein	9% casein
Lumen diameter (μm)	407.7 ± 15.7 (10)	388.0 ± 14.1 (11)
Maximal contraction		
125 mM KPSS (mN/mm)	6.01 ± 0.35	6.27 ± 0.25 (11)
KCl (mN/mm)	3.27 ± 0.26 (9)	3.11 ± 0.22 (9)
Phenylephrine (% 125 mM KPSS)	110.2 ± 5.1 (10)	114.9 ± 2.4 (10)
Maximal relaxation (%)		
ACh	75.3 ± 3.5 (7)	70.0 ± 6.2 (6)
100 μM L-NAME + ACh	20.9 ± 5.4* (5)	27.6 ± 11.7* (6)
VEGF	63.8 ± 6.4 (10)	45.8 ± 4.3§ (11)
10 μM INDO + VEGF	33.2 ± 7.2** (9)	22.4 ± 6.2** (10)
10 μM INDO + L-NAME + VEGF	12.8 ± 3.3¶ (7)	18.9 ± 3.7 (10)
pEC_{50} (−log M)		
KCl	1.45 ± 0.01 (9)	1.46 ± 0.01 (9)
Phenylephrine	6.12 ± 0.08 (10)	6.35 ± 0.14 (10)
VEGF	N/A	N/A

Values are given as mean ± SEM. * $p < 0.05$ *vs* ACh in the same group; § $p < 0.05$ *vs* maximal relaxation to VEGF in 18% casein group; ** $p < 0.05$ *vs* VEGF in the same group; ¶ $p < 0.05$ *vs* VEGF + INDO in the same group. N/A, calculation of pEC_{50} was not appropriate. (*n*), number of observations.

striction tension. The maximal values of the PE and KCl responses were calculated by least squares nonlinear regression analysis, and the pEC_{50} was calculated (Prism 3.0, Graph Pad Software Inc., San Diego, CA, U.S.A.) if appropriate. These values were compared by Mann-Whitney *U* test (SPSS 9.0J, SPSS Inc., Chicago, IL, U.S.A.). If calculation of pEC_{50} was not appropriate, concentration-response curves were compared by two-way repeated-measures ANOVA (Prism 3.0, Graph Pad Software Inc.). Significance was accepted at the level of $p < 0.05$.

RESULTS

Animal Data

The maternal body weight on d 18 or 19 of gestation, the rate of increase of maternal body weight during pregnancy, and the number of fetuses were not different between the 18% and 9% casein groups (Table 2). Averages of fetal and placental weights from each individual litter were not different between the two groups (Table 2).

Vascular Responses

The normalized internal diameters and the maximal tension in the uterine artery induced by 125 mM KPSS were not different between the two groups (Table 3). In the mesenteric artery, there was no significant difference in the KPSS response between groups (18% *versus* 9%, maximal tension, 17.5 ± 0.7 and 18.3 ± 1.00 mN/mm, respectively).

Constriction responses. The uterine artery responses to KCl were not different between groups (Table 3 and Fig. 1*A*). There were also no significant differences in the PE dose-response curve between the groups (Fig. 1*B*). In the mesenteric artery, there was no significant difference in the PE response between groups (data not shown).

Relaxation responses. The maximal relaxation of the uterine artery to ACh (3×10^{-6} M) was not different between the two groups (Table 3 and Fig. 2). In the presence of L-NAME, the maximal relaxation to ACh was significantly reduced in both groups (Table 3 and Fig. 3). VEGF caused relaxation in the uterine artery in both dietary groups, however the overall relaxant response ($p < 0.01$, two-way ANOVA) and maximal relaxation were significantly reduced in the rats fed 9% casein (Table 3 and Fig. 4*A*). VEGF caused significant relaxation of the mesenteric artery in the both dietary groups. Although the effect was less in the 9% casein group, this was not significant (maximal relaxation, $p > 0.05$; two-way ANOVA, $p = 0.05$; Fig. 4*B*). The uterine artery was noted to be more sensitive to VEGF than the mesenteric artery (uterine artery, maximal relaxation attained with 5×10^{-10} M; mesenteric artery,

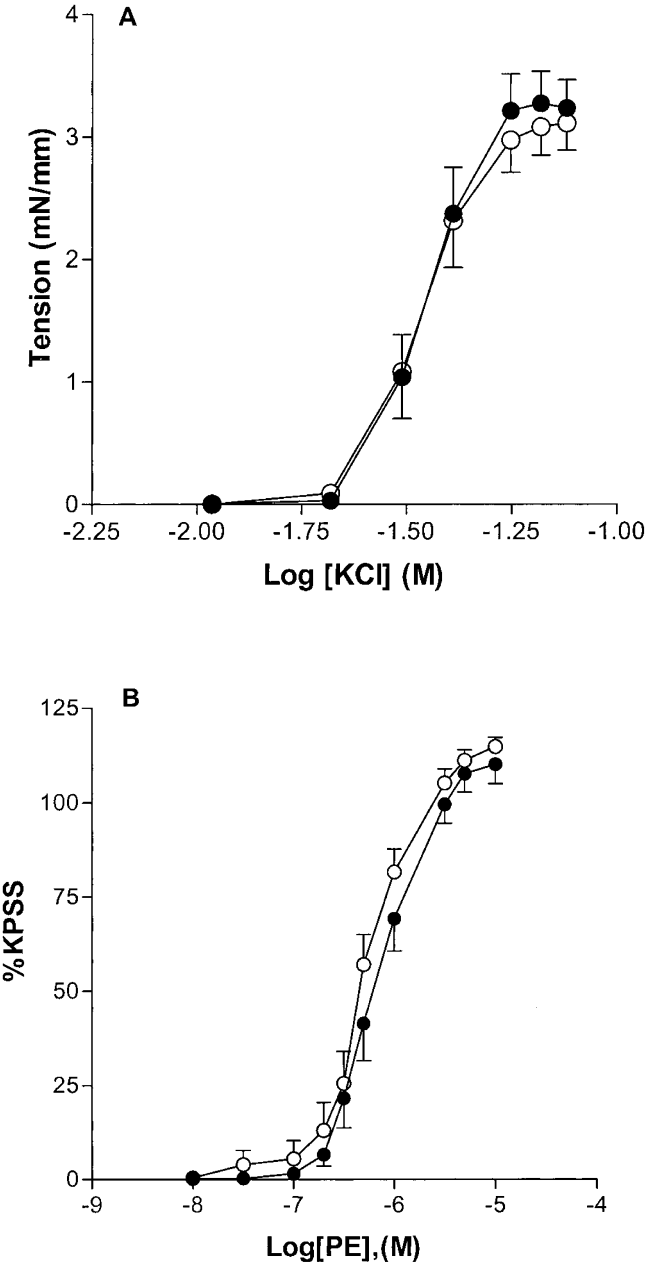

Figure 1. *A*, dose-response curves to KCl of the uterine artery in the 18% (●, $n = 9$) and 9% casein (○, $n = 9$) groups. *B*, dose-response curves to PE of the uterine artery in the 18% (●, $n = 10$) and the 9% casein (○, $n = 10$) groups.

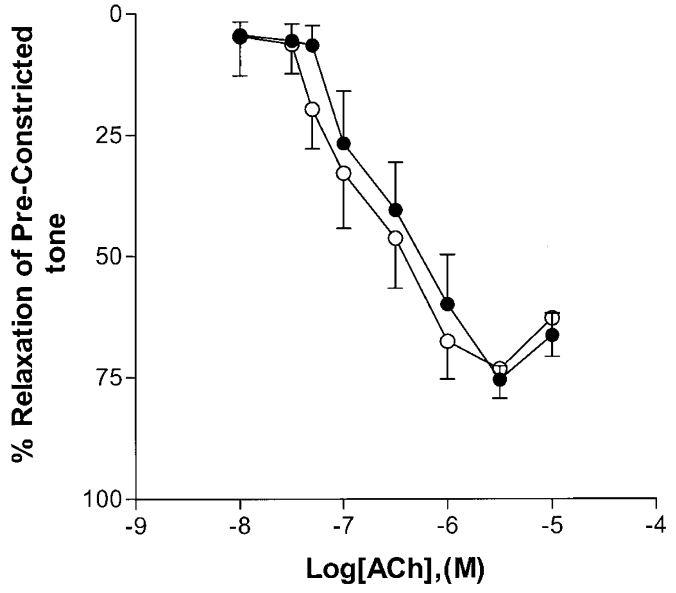

Figure 2. Dose-response curves to ACh of the uterine artery in the 18% (●, $n = 7$) and the 9% casein (○, $n = 6$) groups.

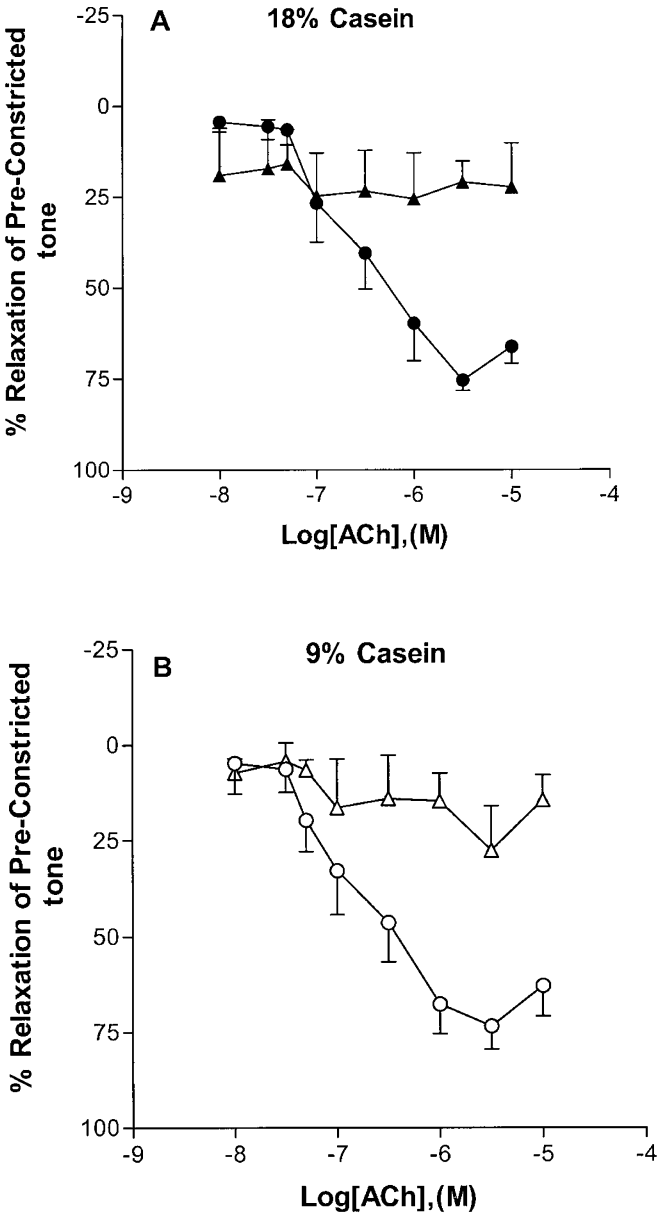

Figure 3. The effect of L-NAME on the response to ACh of the uterine artery in the 18% (●: without inhibitors, $n = 7$; ▲: with L–NAME, $n = 5$) and the 9% casein groups (○: without inhibitors, $n = 6$; △: with L–NAME, $n = 6$).

maximal relaxation attained with 1×10^{-8} M; Fig. 4*C*). At high concentrations the VEGF-induced relaxation was attenuated in the uterine artery (Fig. 4*C*).

In the 18% casein group, the maximal relaxation to VEGF (5×10^{-10} M) in the uterine artery was significantly reduced both by INDO alone ($p < 0.05$ *versus* VEGF) and by INDO and L-NAME together ($p < 0.05$ *versus* VEGF + INDO; Figs. 5*A* and 6*A*). In the 9% casein group, the maximal relaxation to VEGF (1×10^{-9} M) was significantly attenuated by INDO alone ($p < 0.05$ *versus* VEGF), but was not further reduced by INDO and L-NAME together (Figs. 5*B* and 6*B*).

DISCUSSION

Maternal protein restriction in the rat has been associated with hypertension, impaired glucose tolerance, and reduced longevity in adult offspring (4–8). The effects on the pregnant dam have, however, been little studied. Our study has shown that dietary protein restriction is associated with significant blunting of the vasodilatory response of the uterine artery to VEGF. A recent study has also shown that VEGF induces relaxation in rat thoracic aortic rings (20). We have shown for the first time that VEGF dilates the mesenteric artery. However, the sensitivity to VEGF in the mesenteric artery was less than that in the uterine artery, and this is also a novel observation. At high concentrations there is an attenuation of VEGF-induced vasodilation in the uterine artery, not seen in the mesenteric artery. We cannot explain why this attenuation only occurs in the uterine vasculature.

Uterine blood flow is greatly increased in pregnancy, the change being associated with a marked increase in arterial diameter and reversible hypertrophy (21, 22). Enhancement of NO and PGI_2 synthesis as well as remodeling are likely to contribute to the increase in diameter and fall in vascular resistance (23). However, the interaction between a low-protein diet and the physiologic effects in the uterine artery during pregnancy is unknown.

Although earlier reports have documented augmentation of the maximal constriction and sensitivity to PE during rat pregnancy, associated with increased diameter of the uterine artery (24), we did not detect any differences in maximal constriction or sensitivity to PE and KCl between the 18% and the 9% groups. Thus, any differences between the groups do not appear to be related to an increase in vascular smooth muscle cell growth.

Pregnancy is associated with greater serum concentrations of VEGF in humans (15) and VEGF mRNA in the uterine tissue of the rat (17, 18), with an increase in the response to VEGF in the uterine artery (16). It therefore seems reasonable to propose that VEGF plays an important role in dilation of the uterine

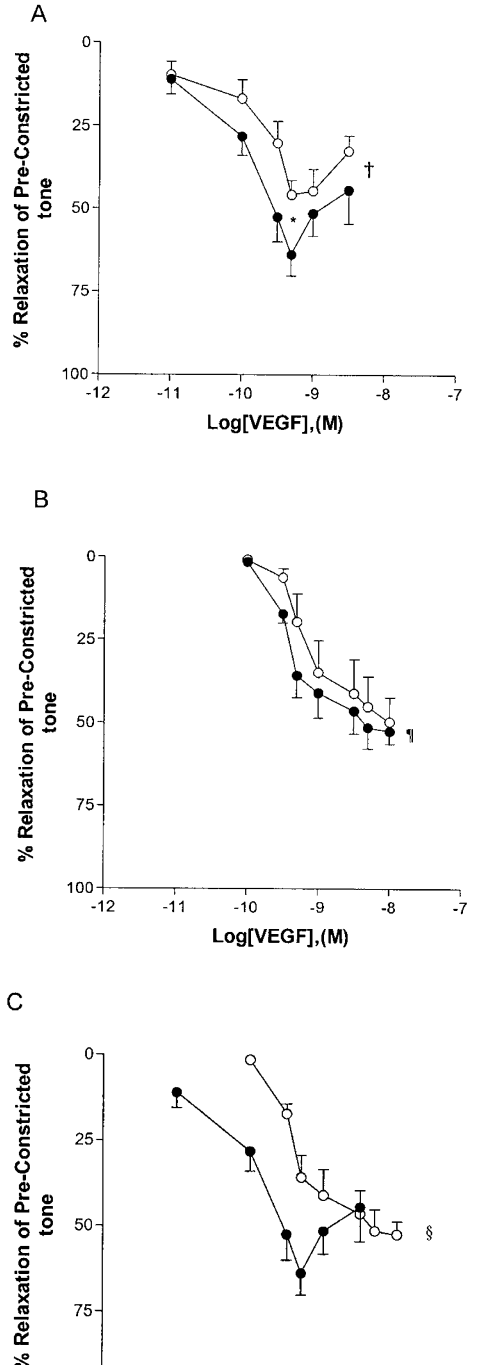

Figure 4. *A*, dose-response curves to VEGF of the uterine artery in the 18% (●, $n = 10$) and the 9% casein (○, $n = 11$) groups. *$p = 0.041$, Mann-Whitney *U* test, for significant difference in the maximal relaxation between the 18% and the 9% casein groups. †$p = 0.004$, significant difference in the overall relaxant response assessed by two-way ANOVA between the 18% and the 9% casein groups. *B*, dose-response curves to VEGF of the mesenteric artery in the 18% (●, $n = 9$) and the 9% casein (○, $n = 8$) groups. ¶$p = 0.05$, difference in the overall relaxant response assessed by two-way ANOVA between the 18% and the 9% casein groups. *C*, dose-response curves to VEGF of the uterine (●, $n = 10$) and mesenteric (○, $n = 9$) artery in the 18% casein group. §$p = 0.004$, significant difference in the overall relaxant response assessed by two-way ANOVA between the VEGF response in the uterine and mesenteric arteries in the 18% casein group.

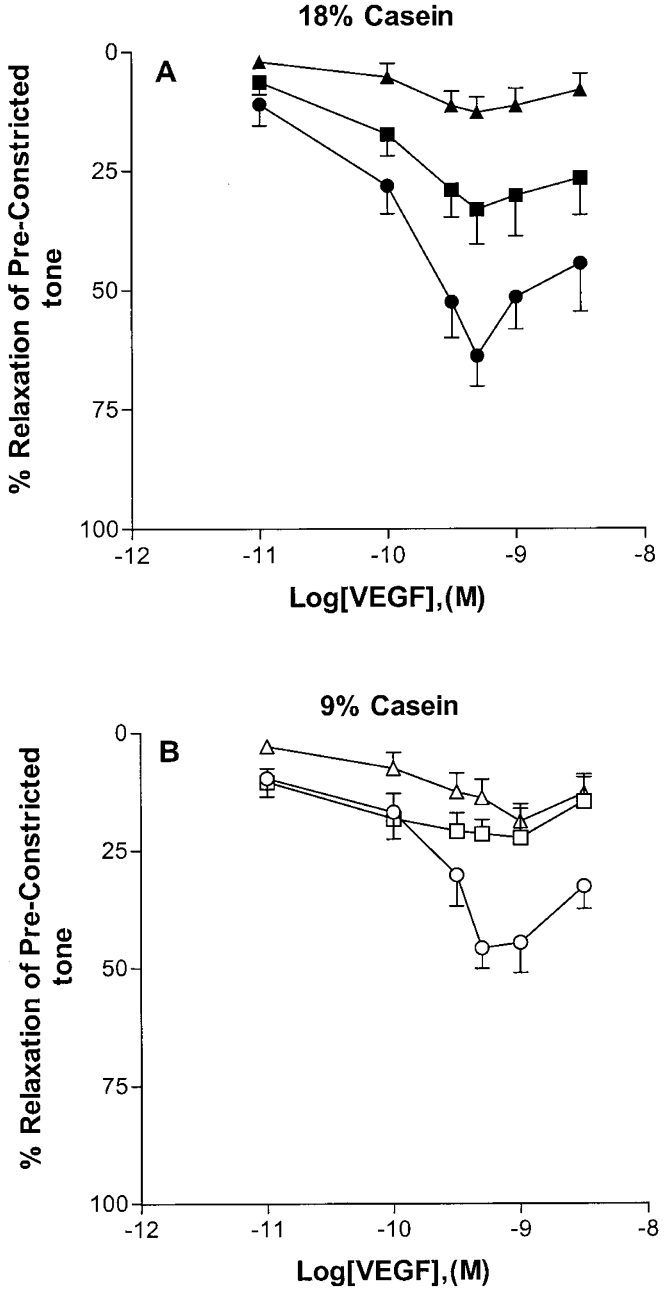

Figure 5. Dose-response curves showing the effects of INDO and combination of INDO and L-NAME on the response to VEGF of the uterine artery in the 18% (*A*, (●: without inhibitors, $n = 10$; ■: with INDO, $n = 9$; ▲: with INDO/L-NAME, $n = 7$) and the 9% casein (*B*, ○: without inhibitors, $n = 11$; □: with INDO, $n = 10$; △: with INDO/L-NAME, $n = 10$) groups.

artery during pregnancy. In addition, the concentration range of VEGF selected included 10^{-10} M VEGF, which could be readily achieved locally under normal physiologic conditions during pregnancy in uterine arteries, and concentrations of this order have been recorded for 10^6 vascular smooth muscle cells in culture (25).

A key finding was the attenuation of maximal relaxation to VEGF in the uterine artery in the 9% casein group compared with the 18% casein group. A study of cultured endothelial cells has shown that the vasodilation to VEGF is induced by PGI_2 and NO via activation of flt-1 and Flk-1/KDR receptors,

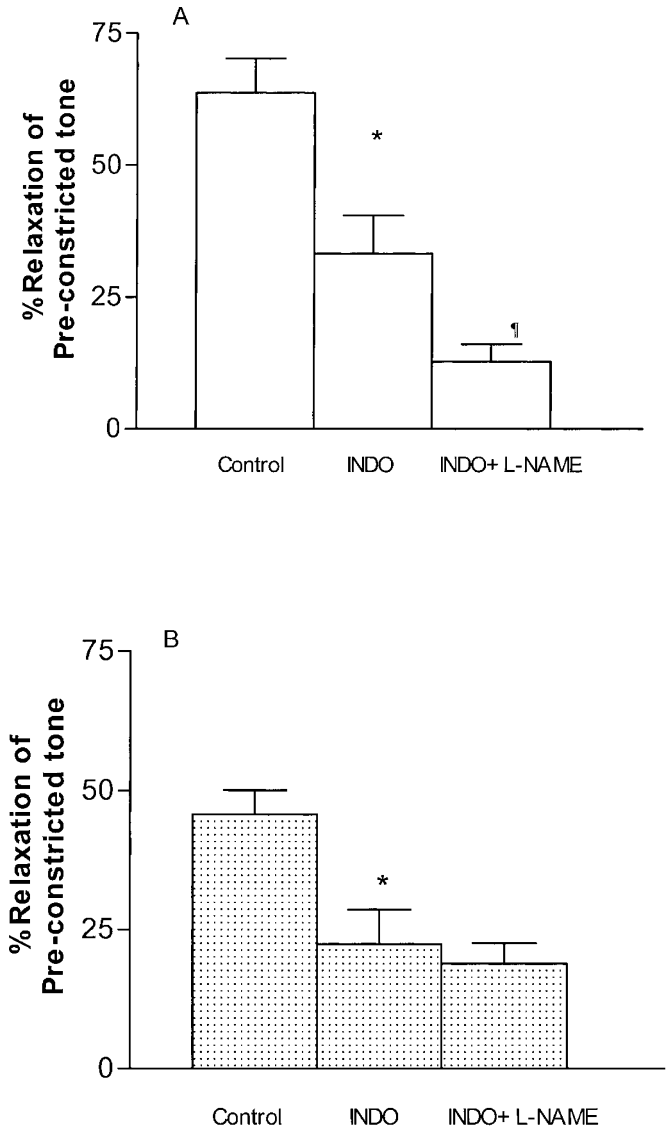

Figure 6. Histogram showing the effect of inhibitors on the maximal VEGF responses in the 18% (*A*; *$p < 0.05$ *versus* control VEGF, ¶$p < 0.05$ *versus* INDO) and the 9% casein groups (*B*, *$p < 0.05$ *versus* control VEGF).

which are specific receptors for VEGF (15). Flt-1 and Flk-1/KDR receptors are present on endothelial cells and on smooth muscle cells in porcine uterine artery (26). The pivotal role of the endothelium was demonstrated by Ni *et al.* (16), who showed complete inhibition of relaxation to VEGF in rat uterine artery vessels denuded of endothelium. Because pregnancy augments the expression of flt-1 and Flk-1/KDR receptors on the endothelial cells in porcine uterine artery (26), the greater vascular response to VEGF in the uterine artery compared with the mesenteric artery observed in this study may be related to an increase in endothelial cell VEGF receptor expression. Among the factors reported to alter VEGF receptor expression, an increase in serum estrogen may play an especially important role in pregnancy (27, 28). In the bovine aorta, VEGF receptor occupation on the endothelium activates phospholipase C, thereby increasing intracellular 1,4,5-inositol trisphosphate and diacylglycerol. The rise in 1,4,5-inositol trisphosphate elevates intracellular calcium, which activates NO synthase, producing NO. The increase in diacylglycerol and intracellular calcium activates protein kinase C and, via subsequent activation of phospholipase A_2, increases PGI_2 (15, 29). In the present study, the maximal relaxation to VEGF in the normal diet group (18% casein) was reduced by INDO, and this reduction was greater than that after superimposed L-NAME. It therefore appears that VEGF-induced PGI_2 production predominates in the rat uterine artery. Like Ni *et al.* (16), we also found an NO-mediated component of VEGF-induced relaxation in the rat uterine artery. However, the magnitude was less in our study, and this contrasting result with that of Ni *et al.* (16) may be attributed to a difference in the experimental protocol used. We used 100 μM L-NAME, whereas Ni *et al.* (16) used 1 mM N^G-nitro-L-arginine. N^G-nitro-L-arginine is known to be a potent nonselective NO synthase inhibitor compared with other arginine analogs (30); therefore, greater NO inhibition would be expected in the study by Ni *et al.* (16). The differences in NO synthase inhibition of VEGF-induced relaxation between studies may thus be attributed to either a difference in concentration or type of inhibitor used.

The inhibition of VEGF-induced relaxation by L-NAME was significantly less in the 9% group, suggesting that reduced NO production may explain the blunted vasodilation to VEGF in the rats fed low-protein diets. ACh produced relaxation of the preconstricted uterine arterial segments, thereby confirming that the endothelium was intact. Interestingly, no change in the response to ACh was noted on the low-protein diet. In a similar study, Koumentaki *et al.* (31) reported that dietary protein restriction attenuated ACh-induced relaxation in small mesenteric arteries. This further confirms our findings that nutritional restriction in pregnancy induces vascular abnormalities that vary between uterine and mesenteric vessels. In the present study, ACh-induced relaxations were greatly reduced with L-NAME, confirming that NO is likely to account for ACh dilation in the uterine artery (32). The contrast with VEGF responses may indicate altered flt-1 and Flk-1/KDR receptor density in the low-protein group as opposed to perturbation in the NO signal transduction pathway or in vascular smooth muscle sensitivity to NO. Inasmuch as a low-protein diet may induce serum estrogen deficiency in rat (33, 34), this may arise from lowering of the plasma concentration of this steroid. Further studies are indicated in which the serum estrogen concentration and receptor density are simultaneously determined.

In summary, we have shown that the vasodilatory response to VEGF is reduced in the uterine artery of pregnant rats fed a low-protein diet, possibly owing to attenuation of the NO component of VEGF-induced vasorelaxation. Preliminary studies further support this hypothesis, indicating that VEGF-induced NO release is reduced in uterine arteries from protein-restricted dams compared with control rats (Brawley, Itoh, and Clough, unpublished observation). Because VEGF is likely to contribute to the increment in uterine blood flow during pregnancy, this attenuation of VEGF dilation might be a factor in the reduction in uterine blood flow that occurs on a globally restricted diet (11). In view of the many reports of adulthood cardiovascular dysfunction in the offspring of rats fed a low-protein diet, and because of the central importance of utero-

placental blood flow in fetal well-being, the data presented here suggest a role for altered uterine artery function in this model of fetal programming.

Acknowledgments. The authors thank Genentech Inc, U.S.A., for kindly supplying the VEGF necessary for these experiments.

REFERENCES

1. Barker DJP, Bull AR, Osmond C, Simmonds SJ 1990 Fetal and placental size and risk of hypertension in later life. BMJ 301:259–263
2. Barker DJP, Gluckman KN, Godfrey JE, Harding JE, Owens JA, Robinson JS 1993 Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938–941
3. Phillips DI, Barker DJP, Hales CN, Hirst S, Osmond C 1994 Thinness at birth and insulin resistance in adult life. Diabetologia 37:150–154
4. Langley SC, Jackson AA 1994 Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. Clin Sci 86:217–222
5. Langley SC, Gardner DS, Jackson AA 1996 Association of disproportionate growth of fetal rats in late gestation with raised systolic blood pressure in later life. J Reprod Fertil 106:307–312
6. Hales CN 1997 Fetal and infant growth and impaired glucose tolerance in adulthood: the "thrifty phenotype" hypothesis revisited. Acta Paediatr Suppl 442:73–77
7. Ozanne SE, Wang CL, Dorling MW, Petry CJ 1999 Dissection of the metabolic actions of insulin in adipocytes from early growth-retarded male rats. J Endocrinol 162:313–319
8. Sherman RC, Langley-Evans SC 2000 Antihypertensive treatment in early postnatal life modulates prenatal dietary influences upon blood pressure in the rat. Clin Sci 98:269–275
9. Ozaki T, Nishina H, Hanson MA, Poston L 2001 Dietary restriction in pregnant rats causes gender-related hypertension and vascular dysfunction in offspring. J Physiol (Lond) 530:141–152
10. Reynolds LP, Redmer DA.1995 Utero-placental vascular development and placental function. J Anim Sci 73:1839–1851
11. Ahokas RA, Reynolds SL, Anderson GD, Lipshitz J 1984 Maternal organ distribution of cardiac output in the diet-restricted pregnant rat. J Nutr 114:2262–2268
12. Vaisman N, Gospodarowicz D, Neufeld G 1990 Characterization of the receptors for vascular endothelial growth factor. J Biol Chem 265:1941–1946
13. Shifren JL, Doldi N, Ferrara N, Mesiano S, Jaffe RB 1994 In the human fetus, vascular endothelial growth factor is expressed in epithelial cells and myocytes, but not vascular endothelium: implication for mode of action. J Clin Endocrinol Metab 79:316–322
14. Peters KG, Vries CD, Williams LT 1993 Vascular endothelial growth factor receptor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. Proc Natl Acad Sci USA 90:8915–8919
15. He H, Venema VJ, Gu X, Venema RC, Marrero MB, Caldwell RB 1999 Vascular endothelial growth factor signals endothelial cell production of nitric oxide and prostacyclin through Flk/KDR activation of c-Src. J Biol Chem 274:25130–25135
16. Ni Y, May V, Braas K, Osol G 1997 Pregnancy augments uteroplacental vascular endothelial growth factor gene expression and vasodilator effects. Am J Physiol 273:H938–H944
17. Evans PW, Wheeler T, Anthony FW, Osmond C 1998 A longitudinal study of maternal serum vascular endothelial growth factor in early pregnancy. Hum Reprod 4:1057–1062
18. Bosio PM, Wheeler T, Anthony F, Conroy R, O'Herlihy C, McKenna P 2001 Maternal plasma vascular endothelial growth factor concentrations in normal and hypertensive pregnancies and their relationship to peripheral vascular resistance. Am J Obstet Gynecol 184:146–152
19. Mulvany MJ, Halpern W 1977 Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 41:19–26
20. Liu MH, Jin HK, Floten HS, Yang Q, Yin AP, Furnary A, Zioncheck TF, Bunting S, He GW 2001 Vascular endothelial growth factor-mediated endothelium-dependent relaxation is blunted in spontaneous hypertensive rats. J Pharmacol Exp Ther 296:473–477
21. Cipolla M, Osol G 1994 Hypertrophic and hyperplastic effects of pregnancy on the rat uterine arterial wall. Am J Obstet Gynecol 171:805–811
22. Keyes LE, Moore LG, Walchak SJ, Dempsey EC 1996 Pregnancy-stimulated growth of vascular smooth muscle cells: importance of protein kinase C–dependent synergy between estrogen and platelet-delivered growth factor. J Cell Physiol 166:22–32
23. Poston L, McCarthy AL, Ritter JM 1995 Control of vascular resistance in the maternal and feto-placental arterial beds. Pharmacol Ther 65:215–239
24. St-Louis J, Paré H, Sicotte B, Brochu M 1997 Increase reactivity of rat uterine arcuate artery throughout gestation and postpartum. Am J Physiol 273:H1148–H1153
25. Bausero P, Ben-Mahdi M-H, Mazucatelli J-P, Bloy C, Perrot-Applanat M 2000 Vascular endothelial growth is modulated in vascular muscle cells by estradiol, tamoxifen, and hypoxia. Am J Physiol 279:H2033–H2042
26. Winther H, Ahmed A, Dantzer V 1999 Immunohistochemical localization of vascular endothelial growth factor (VEGF) and its two specific receptors, Flt-1 and KDR, in the porcine placenta and non-pregnant uterus. Placenta 20:35–43
27. Sugino N, Kashida S, Takiguchi S, Karube A, Kato H 2000 Expression of vascular endothelial growth factor and its receptors in the human corpus luteum during the menstrual cycle and in early pregnancy. J Clin Endocrinol Metab 85:3919–3924
28. Storment JM, Meyer M, Osol G 2000 Estrogen augments the vasodilatory effects of vascular endothelial growth factor in the uterine circulation of the rat. Am J Obstet Gynecol 183:449–453
29. Xia P, Aiello LP, Ishii H, Jiang ZY, Park DJ, Robinson GS, Takagi H, Newsome WP, Jirousek MR, King GL 1996 Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth. J Clin Invest 98:2018–2026
30. Vargas HM, Cuevas JM, Ignarro LJ, Chaudhuri G 1991 Comparison of the inhibitory potencies of N^G-methyl-, N^G-nitro- and N^G-amino-L-arginine on EDRF function in the rat: evidence for continuous basal EDRF release. J Pharmacol Exp Ther 257:1208–1215
31. Koumentaki A, Anthony FW, Poston L, Wheeler T 2001 A low protein diet impairs vascular relaxation in virgin and pregnant rats. Clin Sci (in press)
32. Jovanovic A, Jovanovic S, Grbovic L 1997 Endothelium-dependent relaxation in response to acetylcholine in pregnant guinea-pig uterine artery. Hum Reprod 12:1805–1809
33. González CG, Garcia FD, Fernández SF, Patterson AM 1997 Role of 17-β-estradiol and progesterone on glucose homeostasis: effects of food restriction (50%) in pregnant and non pregnant rats. J Endocrinol Invest 20:397–403
34. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R 2000 Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and estrogen deficiency. J Bone Miner Res 15:683–690