Effects of dietary L-arginine on structure and function of flow-restricted vein grafts

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Purpose: Experiments were designed to determine effects of dietary supplementation with L-arginine on structure and function of flow-restricted vein grafts.
Methods: Saphenous veins were placed as bilateral interposition grafts in femoral arteries of two groups of adult male mongrel dogs; one group was maintained on a normal diet (control), the other group supplemented with L-arginine (200 mg/kg per day) beginning 1 week before surgery. In each dog, flow was reduced by 50% in one graft by placing an adjustable clamp on the artery distal to the distal anastomosis. Plasma amino acids and oxidized products of nitric oxide (NO_x) were measured before and after L-arginine feeding. At postoperative week 4, grafts were removed and prepared for organ chamber studies to determine functions of the endothelium or smooth muscle and for histology.
Results: Plasma L-arginine increased within 3 hours after feeding and increased from 141 ± 8 nmol/mL to 169 ± 11 nmol/mL (n = 6) after 5 weeks of supplementation. Plasma ornithine and citrulline paralleled arginine, whereas circulating NO_x was unchanged. Maximal contractions to 60 mmol/L KCl were reduced in grafts from L-arginine–fed dogs. Endothelium-dependent relaxations to the calcium ionophore A23187 and relaxations of the smooth muscle NO were reduced in grafts from L-arginine–fed dogs. Neointimal hyperplasia was increased in grafts with reduced flow and not affected by arginine feeding.
Conclusions: Dietary supplementation with L-arginine did not increase plasma NO in dogs with peripheral vein grafts or increase endothelium-dependent relaxations in control or flow-restricted grafts. Therefore, dietary supplementation with L-arginine may not improve long-term functions of flow-restricted peripheral bypass grafts. (J Vasc Surg 2001;33:829-39)

Development of intimal hyperplasia in autogenous vein grafts is a major factor limiting long-term patency of the grafts.[1,2] The ability to control or manipulate morphologic and functional changes associated with development of intimal hyperplasia in vein grafts is of obvious clinical importance. One such experimental manipulation is dietary supplementation with the precursor in nitric oxide (NO). In the setting of hypercholesterolemia, dietary supplementation with L-arginine decreases development of intimal hyperplasia and improves vein graft reactivity.[3-6] This antiatherogenic effect is thought to be mediated through NO and can be blocked by L-NAME, an analog of L-arginine and inhibitor of NO synthesis.[7] Dietary supplementation with L-arginine affects other cells, which may also have an impact on neointimal formation including enhancing lymphocyte immunoresponsiveness and inhibiting platelet aggregation and monocyte-endothelial adhesiveness.[8-10] However, not all studies have shown a beneficial effect of dietary supplementation with L-arginine on response to vascular injury.[11] The magnitude of these effects may be related to the presence of hypercholesterolemia, duration of supplementation, and the type of vascular injury.[12-16]

In addition to its role as a precursor for NO, L-arginine may also be metabolized by arginine decarboxylase to agmatine, an inhibitor of NO synthase,[17-19] and by arginase to form ornithine, a precursor in polyamine synthesis and an important component in vascular remodeling[20-22] (Fig 1). In addition, L-arginine can act directly on adenosine triphosphate–sensitive potassium channels, affecting changes in vascular reactivity that are independent of NO formation.[23]

Given the multiple physiologic effects of L-arginine and its availability as a commercial food supplement, it is important to define whether dietary supplementation with L-arginine affects other types of vascular remodeling in the absence of hypercholesterolemia. Changes in blood flow modulate development of intimal hyperplasia in vein grafts.[24] Experiments of the current study were designed to determine effects of dietary L-arginine on development of intimal hyperplasia in vein grafts under normal and flow-compromised conditions in the absence of hypercholesterolemia in dogs.

MATERIALS AND METHODS

Animal model. Adult male mongrel dogs (24-26 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg; supplemental doses were given as needed). Animal care was in compliance with the *Principles of Laboratory Animal Care* formulated by the National
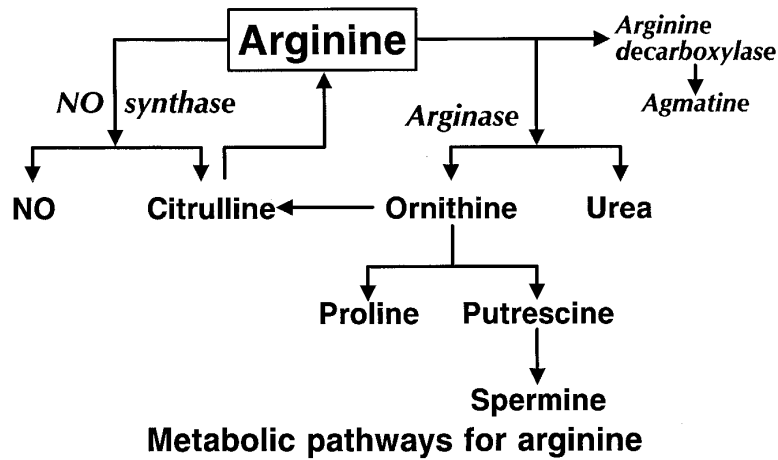
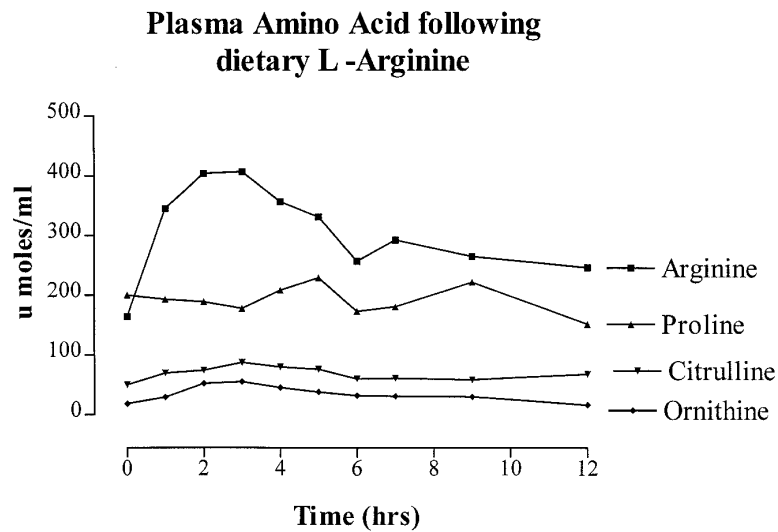**Fig 1.** Schematic of possible metabolites of L-arginine.

Fig 2. Time course of mean changes in plasma arginine, proline, citrulline, and ornithine after arginine feeding (200 mg/kg per day) (n = 3-7, samples from different dogs for each data point). Plasma arginine peaked 3 hours after feeding, increasing from 164 ± 11.2 to 407.7 ± 18.9 μmol/L. Parallel increases at 3 hours were also seen in plasma ornithine (18.3 ± 1.3 to 55 ± 2.6 μmol/L) and citrulline (49.8 ± 8.1 to 87.4 ± 6.68 μmol/L).

Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication no. 86-23, revised 1996). Penicillin G benzathine suspension (1.2 million units) was given intramuscularly as prophylaxis against wound infection.

Two groups of dogs were studied. One group (control, n = 12) received a control diet, and the second group (arginine fed, n = 12) received a diet supplemented with L-arginine at a dose of 200 mg/kg per day. Arginine feeding began 1 week before surgery. In each dog, autologous saphenous veins were placed bilaterally as reversed inter-position grafts in the femoral arteries with standard surgical aseptic technique. All vascular anastomoses were performed in an end-to-end fashion with 6-0 monofilament polypropylene sutures. Once the grafts were in place, flow was measured with an ultrasonic flow probe (Transonic, Inc, Ithaca, NY). In one graft of each dog, flow was decreased 50% by an adjustable clamp placed on the femoral artery 1 cm distal to the distal anastomosis. All dogs received aspirin (325 mg/d) throughout the 4 weeks after surgery, beginning the first postoperative day.

Plasma analysis. Blood samples were taken from cephalic veins at the following time points: before L-arginine supplementation, hourly for up to 12 hours after feeding,
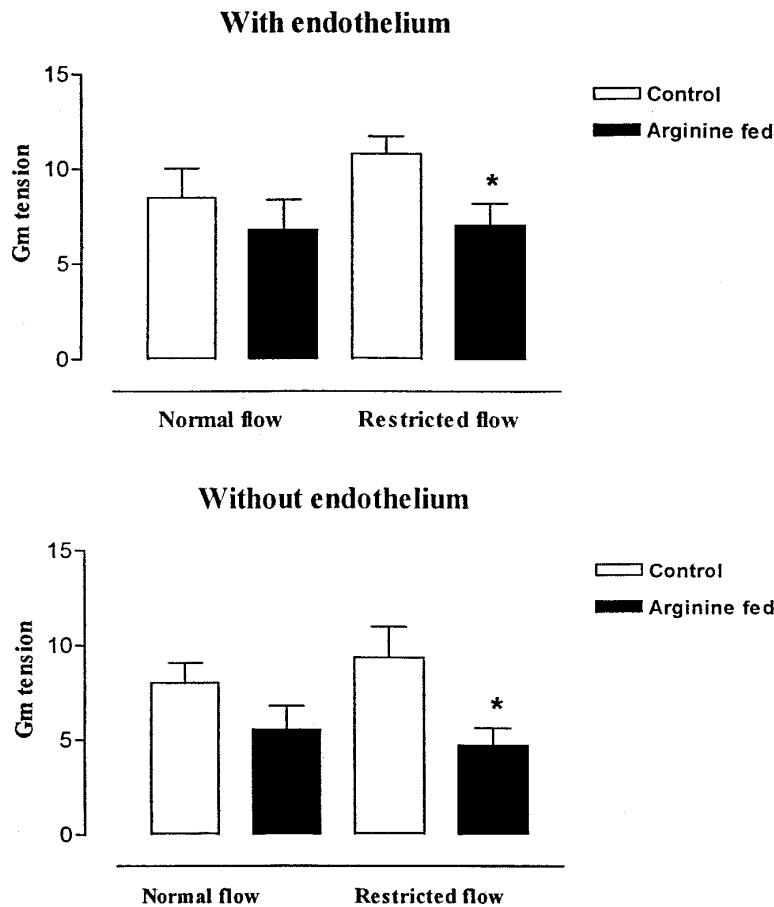
With endothelium

Without endothelium

Fig 3. Contractions to 60 mmol/L KCl in saphenous vein grafts from control and arginine-fed dogs. Data are presented as mean ± SEM; n = 6 for each group. A significant decrease in contractions was observed between control and arginine-fed dogs in grafts with (10.8 ± 0.9 vs 7.1 ± 1.2 gm tension) and without (9.4 ± 1.7 vs 4.8 ± 0.9 gm tension) endothelium under conditions of restricted flow. *Asterisks* denote statistically significant difference from contractions of grafts from control dogs by analysis of variance ($P < .05$).

at the time of surgery, 1 week after surgery, and at the time grafts were removed for study (week 5 of arginine feeding, postoperative week 4). Plasma from these blood samples was used for measurement of oxidized products of NO (NO_x) and amino acids. Amino acid levels were determined with ion exchange chromatography by the use of an established protocol on a Beckmann 6300 (Beckmann Coulter, Inc, Fullerton, Calif) by the blood chemistry laboratory at the Mayo Clinic, Rochester. NO_x was measured at 85°C with chemiluminescence (Sievers Nitric Oxide Analyzer, Model 270B; Sievers Instruments, Boulder, Colo).[25] Plasma (100 μL) was injected into a sampling receptacle containing 5 mL of 0.1 mol/L vanadium III chloride in 3 mol/L hydrochloric acid. At 85°C NO_x reduces to NO when exposed to vanadium III.[26] NO_x was measured twice for each sample and averaged to obtain one data point. If duplicate values differed by more than 10,000 mV/s, then a third sample was analyzed.

In vitro studies. Four weeks after surgery, animals were anesthetized (sodium pentobarbital, 30 mg/kg,

intravenously), and blood flow, vessel diameter, and cross-sectional area were measured through all grafts with Doppler scanning (Apogee RX400; Interspec, Ambler, Pa). The grafts were then removed and placed in chilled modified Kreb's Ringer bicarbonate solution of the following composition: NaCl, 118.3 mmol/L; KCl, 4.7 mmol/L; $MgSO_4$, 1.2 mmol/L; KH_2PO_4, 1.22 mmol/L; $CaCl_2$, 2.5 mmol/L; $NaHCO_3$, 25 mmol/L; and glucose, 11.1 mmol/L (control solution). The grafts were prepared for study in organ chamber experiments immediately after explantation.

Grafts were carefully dissected free of surrounding connective tissue, and the mid portion of the grafts was cut into rings 5 mm in length. The endothelium was removed intentionally from some rings by gently rubbing the luminal surface with a cotton swab wetted with control solution.

Rings were suspended between a fixed point and a force transducer (Gould UC2; Gould Instrument Systems, Inc, Cleveland, Ohio) with two stainless steel wires
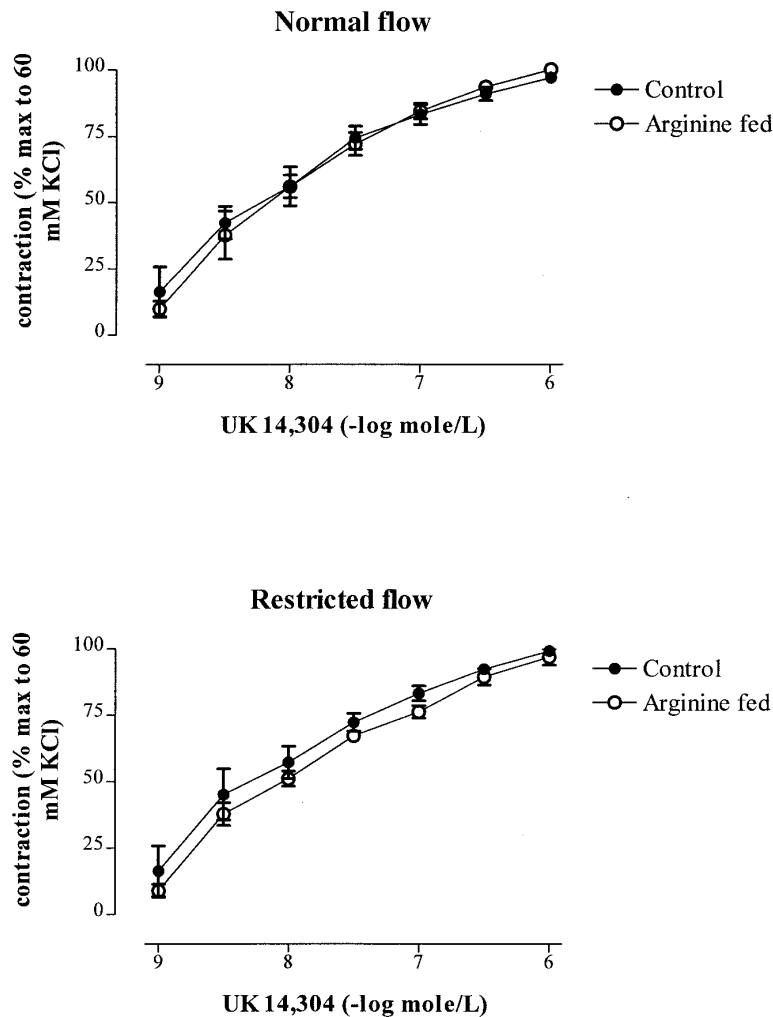
Normal flow

Restricted flow

Fig 4. Contractions to the α_2-adrenergic agonist UK 14,304 in vein grafts without endothelium from control and arginine-fed dogs. Contractions of grafts under normal flow are shown in *upper panel*; those from grafts with restricted flow are shown in the *lower panel*. Values are expressed as percent of maximal contraction to KCl (60 mm). Data are presented as mean ± SEM; n = 6 for each group. Contractions of rings with endothelium were identical to rings without endothelium, which are omitted for clarity.

inserted into the lumen of the vessel for measurement of isometric force. The suspended rings were placed into an organ chamber containing 25 mL of control solution maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Passive tension on each ring was progressively increased and active tension of 20 mmol/L KCl measured until the rings were stretched to the optimal point on their length/tension curve. Once optimal length (baseline tension) was determined, the rings were allowed to equilibrate for 30 minutes, and then contractions to 60 mmol/L KCl were measured.

Rings were washed with control solution and allowed to equilibrate for 40 minutes in the presence of indomethacin (10^{-5} mol/L) to inhibit cyclooxygenase. Responses in the absence and presence of N^G-monomethyl-L-arginine (L-NMMA, 10^{-4} mol/L) to in-

hibit NO synthase were tested on pairs of rings with and without endothelium from the same animals. Once an inhibitor was added to an organ chamber, it remained in the incubation medium for the duration of the experiment. Cumulative concentration-response curves to the α_2-agonist UK 14,304 (10^{-9} to 10^{-6} mol/L) were recorded from baseline tension. The rings then were washed and allowed to equilibrate. After submaximal contraction with prostaglandin $F_{2\alpha}$ (2×10^{-6} mol/L), cumulative concentration-response curves were recorded to the following agents: adenosine diphosphate (10^{-9} to 10^{-4} mol/L), the calcium ionophore A23187 (10^{-9} to 10^{-6} mol/L) in rings with endothelium, and NO (10^{-9} to 10^{-5} mol/L) in rings without endothelium. α_2-Adrenergic responses were tested because these receptors release NO from endothelial cells and receptors
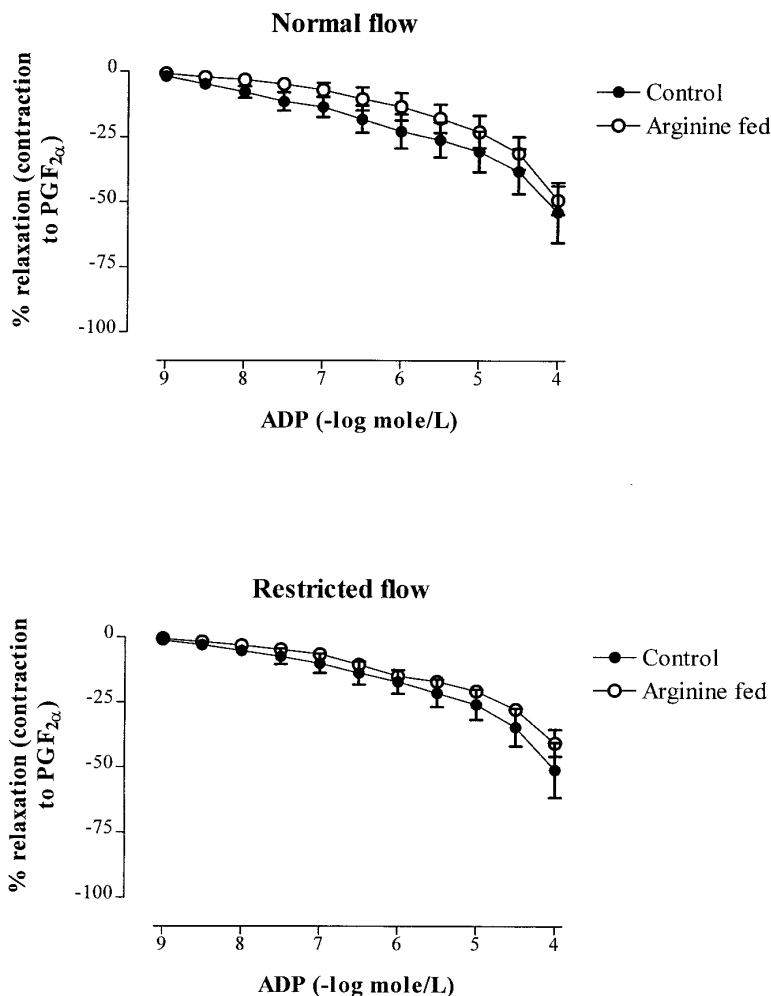
Fig 5. Relaxations to adenosine diphosphate in rings with endothelium in vein grafts with normal flow (*upper panel*) and restricted flow (*lower panel*) from control and arginine-fed dogs. Values are shown as percent change in tension from contractions to prostaglandin $F_{2\alpha}$ (9.2 ± 2.1 and 7.4 ± 1.5 g in rings of grafts with normal flow from control and arginine-fed dogs, respectively; 7.9 ± 2.4 and 8.4 ± 2.5 g in rings of grafts with restricted flow from control and arginine-fed dogs, respectively). Data are expressed as mean ± SEM; n = 5 to 6 per group. Relaxations of rings without endothelium were similar to those with endothelium and are omitted for clarity (n = 5-6 per group).

on the smooth muscle are modified with denervation.[27-29] Acetylcholine, which is usually used as a standard agent to test endothelium in arteries, does not cause consistent relaxation in canine veins, vein grafts, or saphenous veins used as bypass grafts in humans.[24,27,30-33] Therefore, A23187, which causes direct stimulation of NO synthase through a calcium-dependent, receptor-independent mechanism, was used to test the endothelium in the present experiments. Drugs were prepared fresh daily. NO was prepared according to the method of Palmer et al.[34]

Histologic analysis. Functional studies (organ chamber experiments) and histology were performed on each graft. Rings from proximal, middle, and distal sections of grafts adjacent to those used in organ chamber studies were fixed for 24 hours in 10% formalin solution and prepared for light microscopy by the histology laboratory at the Mayo Clinic. Adjacent sections (5 μm each) were stained for elastin van Gieson to differentiate connective tissue from smooth muscle and endothelial cells and bone sialoprotein with immunohistochemistry. Intimal hyperplasia was quantified on sections stained with elastin van Gieson with a digitizer pad and IBAS image analysis software (Kontron Electronics, Munich, Germany). This system allows for a marker to trace the exact circumference of luminal surface and intimal/media borders. The average thickness of neointima was calculated by measuring the area of the neointima and dividing by the circumference of the internal elastic lamina because the distinction between the media and adventitia is not easily demarcated in all specimens.[24,30,33]

Adjacent sections of grafts were stained immunohistochemically for bone sialoprotein (BSP, polyclonal rab-
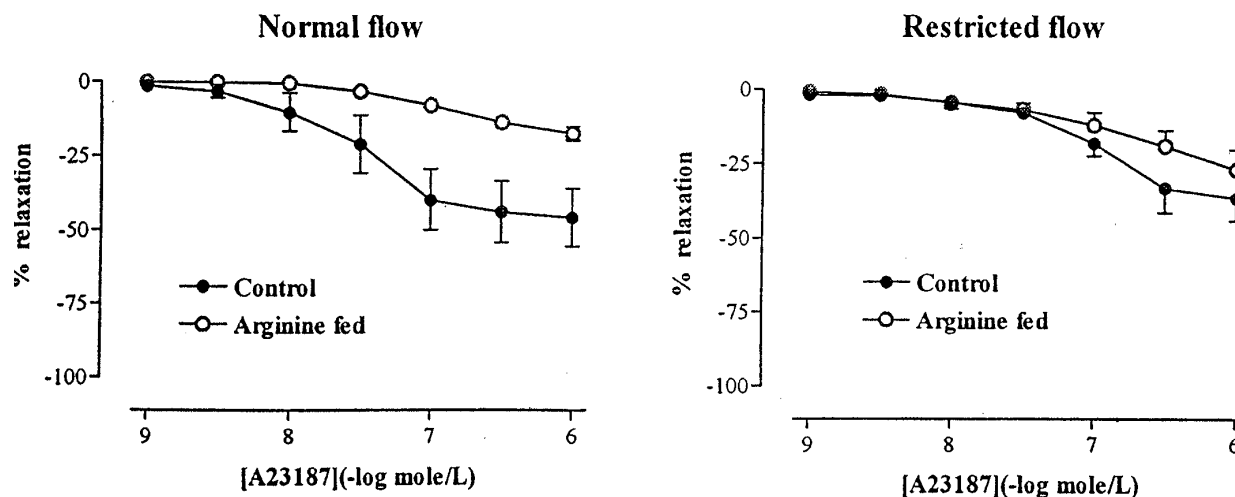
Normal flow

Restricted flow

Fig 6. Responses to the calcium ionophore A23187 in vein grafts with endothelium from control and arginine-fed dogs under conditions of normal flow (*left panel*) and restricted flow (*right panel*). Values are expressed as percent of relaxations from contraction to prostaglandin $F_{2\alpha}$ (2×10^{-6} mol/L; 2.5 ± 0.7 and 1.3 ± 0.5 g in rings from control and arginine-fed dogs, respectively). Data are expressed as mean ± SEM; n = 6 for each group. A significant decrease in maximal relaxation was observed in grafts from arginine-fed dogs with normal flow ($P = .0178$).

bit, LF-100; gift from Dr Larry Fisher, National Institutes of Dental Research, National Institutes of Health, Bethesda, Md). Vectastatin ELITE ABC kit (Vector Laboratories, Burlingame, Calif) was used for sections stained for BSP. Hematoxylin was used as counterstain for all other antibodies. Adjacent sections were stained with immunoglobulin G in the absence of primary antibody to control for nonspecific staining. Immunostaining was performed in the immunohistochemical laboratory and bone morphometric laboratory at the Mayo Clinic. In general, paraffin was removed from the tissue sections with xylene (100%). Tissue was rehydrated into descending ethanols and blocked in Tris-buffered saline (0.05 mol/L Tris, 0.01% bovine serum albumin, 0.9% NaCl, pH 7.5) containing 0.3% casein and 10% normal goat serum. Incubations with primary and secondary antibody (biotinylated goat anti-rabbit) were performed at room temperature followed by two 15-minute washes in Tris-buffered saline containing 0.02% Triton X-100. Endogenous peroxidase activity was inhibited with 0.01% H_2O_2. Sections were rinsed with tap water, dehydrated with ascending alcohols, and cleared with xylene.

Calculations and statistical analysis. All data are expressed as mean ± SEM. The *n* represents the number of dogs from which grafts were taken. In the organ chamber experiments, maximal responses were compared among grafts with normal and restricted flow in control and arginine-fed dogs with one-way analysis of variance. When a significant F value was found, Bonferroni's post hoc analysis was used to identify significant differences among means. A Student *t* test for paired observations was used to compare responses between grafts with normal and restricted flow within the diet groups, that is, grafts from dogs fed either a control or L-arginine–supplemented diet. A Student *t* test

for unpaired observations was used to compare differences in responses of grafts between the two diet groups of control and L-arginine–fed dogs. A *P* value less than .05 was considered to be statistically significant.

RESULTS

Graft patency and blood flow

Two grafts with flow restriction were occluded at time of harvest, one in a control and one in an arginine-fed dog. In the remaining grafts, blood flow in flow-restricted grafts averaged 50% less than in unmanipulated grafts (normal flow, 111.5 mL/min, n = 24).

Plasma amino acids

Plasma arginine increased within 3 hours after feeding, increasing from 141.5 ± 8.1 μmol/L to 407.7 ± 18.9 μmol/L. Parallel increases in plasma levels of ornithine (18.3 ± 1.3 to 55 ± 2.6 μmol/L) and citrulline (49.8 ± 8.1 to 87.4 ± 6.68 μmol/L; Fig 2) occurred within the same time period. There were no significant increases in baseline plasma levels of L-arginine after 1 week of feeding. However, baseline plasma L-arginine (141.5 ± 8.1 μmol/L) increased by 19.9% (169.7 ± 11.4 μmol/L, n = 6) after 2 weeks of dietary supplementation and did not increase thereafter. Plasma NO_x (7.1 ± 1.4 nmol/L) did not change significantly after 5 weeks of dietary supplementation with L-arginine (4.9 ± 0.5 nmol/L at week 5, n = 4).

Organ chamber studies

In grafts with normal flow, maximal contractions to KCl were similar in rings with and without endothelium from both control and arginine-fed dogs. However, in grafts with restricted flow, contractions of rings with and without
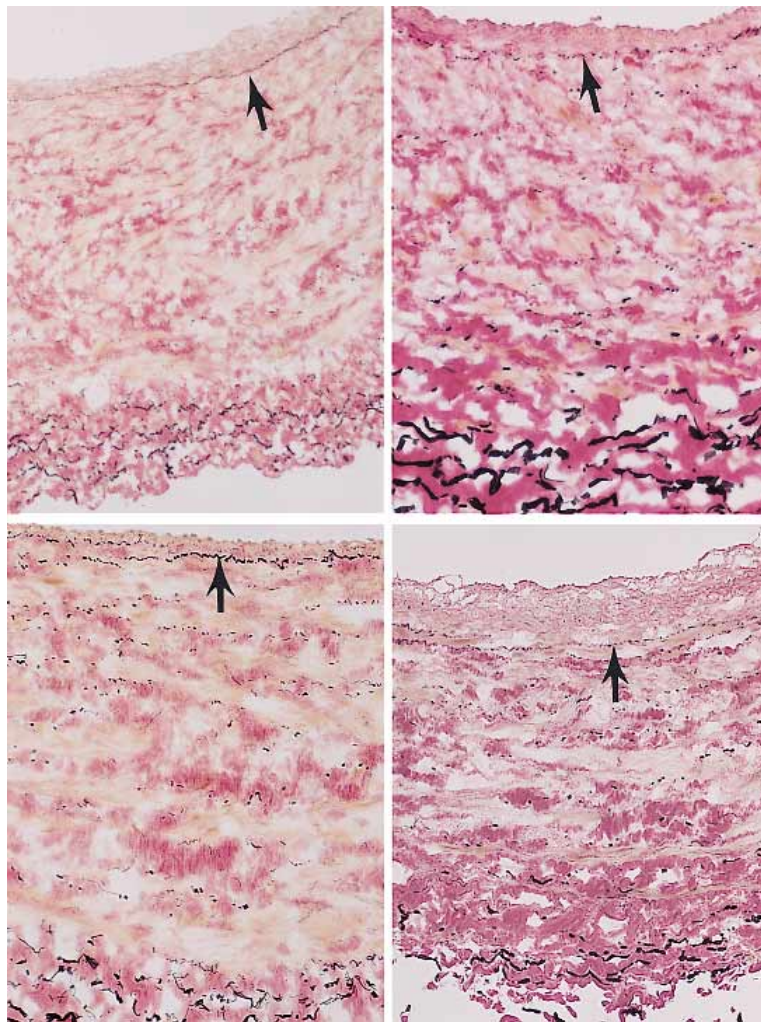
Fig 7. Representative light micrographs of sections stained with elastin van Gieson from the mid portion of normal flow (*top panels*) and flow-restricted (*lower panels*) grafts from control (*left panels*) and arginine-fed (*right panels*) dogs. Intimal hyperplasia was measured as area above intimal elastin lamina (↑). *Black stain*: elastin; *red stain*: collagen; *yellow stain*: other cellular material. Original magnification ×200; sections 5 μm.

endothelium to KCl were significantly less in grafts from arginine-fed dogs compared with control dogs (Fig 3).

UK 14,304. The α_2-adrenergic agonists caused similar concentration-dependent increases in tension in rings with and without endothelium from control and arginine-fed dogs. Contractions were similar for normal and reduced flow grafts (Fig 4). Contractions were unaffected by L-NMMA in grafts with and without endothelium from control and arginine-fed dogs (data not shown, n = 6 per group).

Adenosine diphosphate. Adenosine diphosphate caused comparable relaxations in normal and flow-restricted grafts with and without endothelium (Fig 5). Relaxations were not altered significantly by incubation in L-NMMA (data not shown, n = 6 per group).

A23187. Maximal relaxations to the calcium ion-ophore A23187 in rings with endothelium of normal flow grafts were significantly less in grafts from arginine-fed dogs compared with those from control dogs (Fig 6). In the presence of L-NMMA (10^{-4} mol/L), maximal relaxations to the ionophore in rings from dogs fed a control diet were reduced to that of arginine-fed dogs. L-NMMA did not alter maximal relaxations of rings from arginine-fed dogs.

Nitric oxide. Maximal relaxations to NO were similar (P = .25) in grafts without endothelium under normal flow (59.7% ± 13.4% and 41.8% ± 8.1% in control, n = 5, and arginine-fed dogs, n = 6, respectively) and restricted flow conditions (55.8% ± 16.6% and 43.9% ± 11.1% in control, n = 5, and arginine-fed dogs, n = 6, respectively). Relaxations to NO were not altered significantly by L-NMMA in any group.

Intimal Thickness in
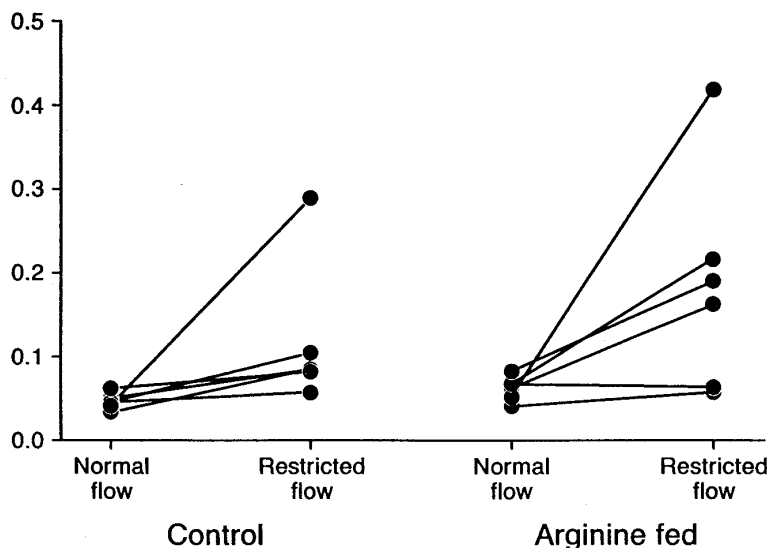# Canine Saphenous Vein Grafts

Fig 8. Intimal hyperplasia was quantified with computer-assisted analysis of light microscopy, measuring the total area of the neointima and dividing by the circumference of the internal elastic lamina. There was a statistically significant increase in intimal hyperplasia in grafts under restricted flow conditions in both control and arginine-fed dogs. There was a trend toward increased development of intimal hyperplasia in arginine-fed dogs compared with control dogs under condition of flow restriction ($P > .05$). Data are shown as measurement from grafts of individual animals. The *line* indicates measurements from control and restricted grafts from the same animal.

Histologic study

Neointimal hyperplasia tended to increase with flow restriction in grafts from both control and arginine-fed dogs (Figs 7 and 8). Grafts from arginine-fed dogs showed less organization of the medial smooth muscle and less staining for bone sialoprotein (Figs 7 and 9).

DISCUSSION

Results of these experiments indicate that dietary supplementation with L-arginine in dogs decreases expression of endothelium-dependent relaxations and contraction of the smooth muscle in both normal and flow-restricted vein grafts. This conclusion is supported by decreased endothelium-dependent relaxations to A23187 and decreased maximal contractions to KCl. Decreased endothelium-dependent relaxations with dietary arginine were unexpected and stand in direct contrast to studies with jugular vein grafts in hypercholesterolemic rabbits where dietary L-arginine attenuated intimal hyperplasia and increased endothelium-dependent relaxations.[3-6] One possible mechanism, which may explain this discrepancy, is that L-arginine may be preferentially used by type II NO synthase (inducible), which is a prominent component in the endothelium of hypercholesterolemic rabbits. In the current study, dogs were not hypercholesterolemic, and type II NO synthase is not a prominent component of NO synthase activity in canine endothelium (unpublished observations).

Infusions of L-arginine also have been shown to improve endothelium-dependent vasodilatation in the coronary and systemic circulation of hypercholesterolemic humans.[26,35,36] However, dietary supplementation with L-arginine did not reduce rejection or transplant-associated intimal thickening.[16] Therefore, whether dietary effects of L-arginine improve vascular function may depend on the presence of hypercholesterolemia or the type of vascular injury.

Effects of L-arginine supplementation under nonatherosclerotic conditions are sparse. When dietary supplementation was given to healthy human volunteers, no improvement in endothelium-mediated vasodilatation was observed.[11] However, effects of L-arginine may be dose and time dependent because vasodilatation in healthy humans was closely correlated with plasma concentrations of arginine.[12] However, this latter study in humans examined only acute single dosing regimens.

Another unexpected finding of the current study was that responses to adenosine diphosphate were not modified by the presence of the endothelium. Adenosine diphosphate can cause direct relaxation of the smooth muscle through activation of purinergic receptors[37] and release of endothelium-derived factors from the endothelium, including that to the vasa vasorum.[27,30,37] Relaxations to adenosine diphosphate can be modulated by changes in blood flow[24] in vein grafts. It is unclear as
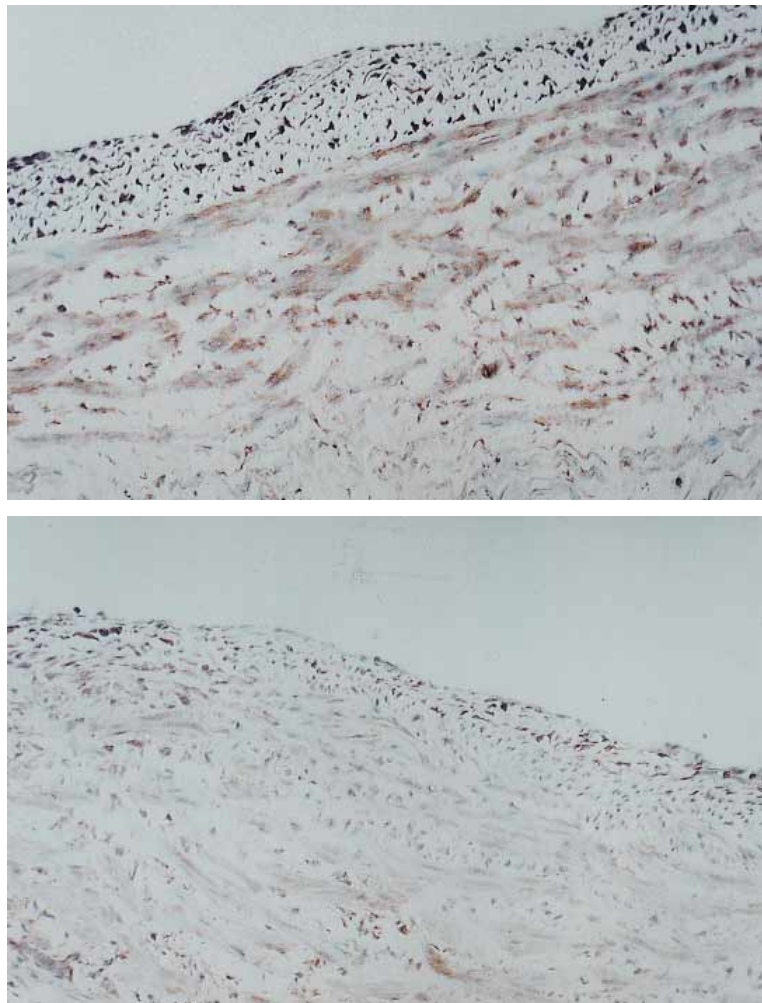
Fig 9. Light micrograph of immunostaining for bone sialoprotein in vein grafts with restricted flow from control dogs (*upper panel*) and arginine-fed dogs (*lower panel*). Staining for bone sialoprotein was greater in control compared with arginine-fed dogs. Original magnification ×100; sections 5 μm.

to why the endothelium-dependent component of the response is lost in the saphenous vein grafts. This may be related to loss of receptors differentially in saphenous compared with femoral vein grafts.

Arginine supplementation reduced contractions to KCl but not to adrenergic stimulation. Arginine may affect functional characteristics of potassium channels, thereby altering membrane potential.[23,38,39] Changes in membrane potential would affect calcium entry by voltage-gated calcium channels and thereby modulate contractions. Stimulation with the α_2-agonist would affect calcium entry by receptor-coupled mechanisms most likely associated with guanine nucleotide regulatory proteins sensitive to pertussis toxin.[40] Alternatively, changes in production of polyamines associated with arginine feeding could also modulate potassium channels in vascular smooth muscle as has been reported for cardiac myocytes.[41]

The observed increases in plasma proline, ornithine, and citrulline within 1 to 2 hours after arginine feeding suggest that dietary supplemented L-arginine may be metabolized by pathways other than NO synthase (Fig 2). Although baseline plasma L-arginine levels increased by 19.9%, sustained parallel increases in plasma NO_x or citrulline were not observed. In excess, L-arginine may be metabolized by arginase leading to formation of polyamines, an important component in remodeling of blood vessels. Polyamines, and specifically the aldehyde metabolite spermine, have an inhibitory effect on the induction of NO synthase[42] and both spermine and spermidine inhibit potassium channels.[41] Although indirect, the possibility of changes in polyamine synthesis is supported by decreases in functional capability of the grafts (decreased contraction to KCl and decreased relaxations to A23187). A more direct approach in support of this

possibility would be to evaluate changes in polyamines within the grafts. In a preliminary study, putrescine, spermine and spermidine were detected by high-pressure liquid chromatography in grafts from arginine-fed (n = 4) and control (n = 1) dogs. Putrescine levels were low, ranging between nondetectable and 6 nmol/mg tissue. Spermine ranged from about 20 to 55 nmol/mg tissue and did not seem to show a consistent pattern of expression with arginine feeding. Spermidine, however, did show differential expression with arginine feeding. Spermidine content in four grafts from arginine-fed dogs averaged 15.1 nmol/mg tissue with a very narrow range of 13.1 to 17.5 nmol/mg tissue. This was about double the spermidine concentration in one graft from a control dog, which measured 8.3 nmol/mg. Although these data are preliminary, they provide support for the speculation that dietary arginine is metabolized by multiple pathways.

Arginine may also be metabolized by arginine decarboxylase leading to production of agmatine, a ligand for imidazoline receptors, a type of α_2-adrenergic receptor and NO synthase regulator.[18,43,44] Unlike responses to the α_2-adrenergic agonist UK 14,304 in coronary arteries from these dogs,[45] contractions to this agonist were not altered by arginine feeding in the grafts. These results, therefore, suggest agonist and vessel specificity in the effects of dietary arginine supplementation.

One important difference between this study and others that have examined effects of L-arginine supplementation is the mode of delivery of the amino acid. In studies with hypercholesterolemic rabbits, rabbits received L-arginine in their drinking water.[3-6] This would imply a sporadic variable dosing. However, clinically L-arginine is commonly prescribed as an oral supplement, which would represent a different pharmacokinetic circulating profile. Oral supplementation has significant effects on the plasma amino acid profile. In addition to increases in plasma L-arginine levels, parallel increases are also seen in asymmetrical N^G, N^G-dimethylarginine (ADMA), an endogenous inhibitor of NO synthase.[10,46] In normal arteries, regenerating endothelial cells have decreased levels of L-arginine and elevated levels of endogenous inhibitors of NO synthase, including ADMA and L-NMMA.[47] This accumulation of endogenous inhibitors of NO synthase and a decrease in the L-arginine/ADMA ratio under conditions of the present experiment may partly explain the decrease in endothelium-dependent relaxation and increased negative effect of arginine feeding on neointimal formations. Although decreases in staining for bone sialoprotein were observed subjectively, it is unclear how this may be related to arginine metabolism specifically or vascular remodeling in general.

In conclusion, results of this study indicate that dietary supplementation with L-arginine in the absence of hypercholesterolemia can decrease contractions and endothelium-dependent relaxations of vein grafts under normal and low flow conditions. These decreases in vein graft reactivity and the tendency toward increased intimal hyperplasia may result from metabolism of L-arginine by enzymes other than NO synthases. Therefore, dietary arginine supplementation to improve vascular function may be indicated only in circumstances where other cardiovascular risk factors like hypercholesterolemia are present.

REFERENCES

1. Clowes AW. Intimal hyperplasia and graft failure. Cardiovasc Pathol 1993;2:179S.
2. Davies MG, Hagen P-O. Pathobiology of intimal hyperplasia. Br J Surg 1994;81:1254-69.
3. Cooke JP, Singer AH, Tsao P, Zera P, Rowan RA, Billingham ME. Antiatherogenic effects of L-arginine in the hypercholesterolemic rabbit. J Clin Invest 1992;90:1168-72.
4. Davies MG, Hagen P-O. Suppression of intimal hyperplasia in experimental vein grafts by oral L-arginine supplementation and single ex-vivo immersion in deferoxamine manganese. J Vasc Surg 1996;23:410-20.
5. Davies MG, Dalen H, Kim JH, Barber L, Svendsen E, Hagen P-O. Control of accelerated vein graft atheroma with the nitric oxide precursor: L-arginine. J Surg Res 1995;59:35-42.
6. Davies MG, Kim JH, Dalen H, Makhoul RG, Svendsen E, Hagen P-O. Reduction of experimental vein graft intimal hyperplasia and preservation of nitric oxide-mediated relaxation by the nitric oxide precursor L-arginine. Surgery 1994;116:557-68.
7. Cayatte AJ, Palacino JJ, Horten K, Cohen RA. Chronic inhibition of nitric oxide production accelerates neointima formation and impairs endothelial function in hypercholesterolemic rabbits. Arterioscler Thromb 1994;14:753-9.
8. Theilmeier G, Chan JR, Zalpour C, Anderson B, Wang BY, Wolf A, et al. Adhesiveness of mononuclear cells in hypercholesterolemic humans is normalized by dietary L-arginine. Arterioscler Thromb Vasc Biol 1997;17:3557-64.
9. Boger RH, Bode-Boger SM, Kienke S, Stan AC, Nafe R, Frolich JC. Dietary L-arginine decreases myointimal cell proliferation and vascular monocyte accumulation in cholesterol-fed rabbits. Atherosclerosis 1998;136:67-77.
10. Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction—its role in hypercholesterolemia. Circulation 1998;98:1842-7.
11. Chin-Dusting JP, Alexander CT, Arnold PJ, Hodgson WC, Lux AS, Jennings GL. Effects of in vivo and in vitro L-arginine e supplementation on healthy human vessels. J Cardiovasc Pharmacol 1996;28:158-66.
12. Bode-Boger SM, Boger RH, Galland A, Tsikas D, Frolich JC. L-arginine -induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. Br J Clin Pharmacol 1998;46:489-97.
13. Cooke JP, Dzau VJ. Derangements of the nitric oxide synthase pathway, L-arginine, and cardiovascular diseases. Circulation 1997;96:379-82.
14. Lou H, Kodama T, Wang YN, Katz N, Ramwell P, Foegh ML. L-arginine prevents heart transplant arteriosclerosis by modulating the vascular cell proliferative response to insulin-like growth factor-I and interleukin-6. J Heart Lung Transplant 1996;15:1248-57.
15. Mugge A, Harrison DG. L-arginine does not restore endothelial dysfunction in atherosclerotic rabbit aorta in vivo. Blood Vessels 1991;28:354-7.
16. Gregory CR, Cooke JP, Patz JD, Berryman ER, Shorthouse R, Morris RE. Enhanced nitric oxide production induced by the administration of L-arginine does not inhibit arterial neointimal formation after overwhelming alloimmune injury. J Heart Lung Transplant 1996;15:58-66.

17. Gonzalez C, Regunathan S, Reis DJ, Estrada C. Agmatine, an endogenous modulator of noradrenergic neurotransmission in the rat tail artery. Br J Pharmacol 1996;119:677-84.

18. Galea E, Regunathan S, Eliopoulos V, Feinstein DL, Reis DJ. Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem J 1996;316:247-9.

19. Grant SK, Green BG, Stiffey-Wilusz J, Durette PL, Shah SK, Kozarich JW. Structural requirements for human inducible nitric oxide synthase substrates and substrate analogue inhibitors. Biochemistry 1998;37: 4174-80.

20. Nakaki T, Kato R. Beneficial circulatory effect of L-arginine. Jpn J Pharmacol 1994;66:167-71.

21. Buga GM, Singh R, Pervin S, Rogers NE, Schmitz DA, Jenkinson CP, et al. Arginase activity in endothelial cells: inhibition by N^G-hydroxy-L-arginine during high-output NO production. Am J Physiol 1996;271:H1988-98.

22. Wang WW, Jenkinson CP, Griscavage JM, Kern RM, Arabolos NS, Byrns RE, et al. Co-induction of arginase and nitric oxide synthase in murine macrophages activated by lipopolysaccharide. Biochem Biophys Res Commun 1995;210:1009-16.

23. Kontos HA, Wei EP. Arginine analogues inhibit responses mediated by ATP-sensitive K+ channels. Am J Physiol 1996;271:H1498-506.

24. Cambria RA, Lowell RC, Gloviczki P, Miller VM. Chronic changes in blood flow alter endothelium-dependent responses in autogenous vein grafts in dogs. J Vasc Surg 1994;20:765-73.

25. Braman RS, Hendrix SA. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium (III) reduction with chemiluminescence detection. Anal Chem 1989;61:2715-8.

26. Panza JA, Casino PR, Badar DM, Quyyumi AA. Effect of increased availability of endothelium-derived nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and patients with essential hypertension. Circulation 1993;87:1475-81.

27. Rhee RY, Gloviczki P, Miller VM. An arginine analog inhibits responses of both the endothelium and smooth muscle of canine in situ vein grafts. Cardiovasc Surg 1997;5:169-75.

28. Miller VM, Barber DA. Modulation of endothelium-derived nitric oxide in canine femoral veins. Am J Physiol 1996;271:H668-73.

29. Flavahan NA, Miller VM, Aarhus LL, Vanhoutte PM. Denervation augments alpha-2 but not alpha-1 adrenergic responses in canine saphenous veins. J Pharmacol Exp Ther 1987;240:589-93.

30. Miller VM, Reigel MM, Hollier LH, Vanhoutte PM. Endothelium-dependent responses in autogenous femoral veins grafted into the arterial circulation of the dog. J Clin Invest 1987;80:1350-7.

31. Luscher TF, Diederich D, Siebenmann R, Lehmann K, Stulz P, von Segesser L, et al. Difference between endothelium-dependent relaxation in arterial and in venous coronary bypass grafts. N Engl J Med 1988;319:462-7.

32. Ku DD, Caulfield JB, Kirklin JK. Endothelium-dependent responses in long-term human coronary artery bypass grafts. Circulation 1991;83:402-11.

33. Clouse WD, Rud KS, Hurt RD, Miller VM. Short-term treatment with transdermal nicotine affects function of canine saphenous veins. Vasc Med 2000;5:75-82.

34. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524-6.

35. Drexler H, Zeiher AM, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolemic patients by L-arginine. Lancet 1991;338:1546-50.

36. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, Cooke JP. L-arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. J Clin Invest 1992;90:1248-53.

37. Houston DS, Shepherd JT, Vanhoutte PM. Adenine nucleotides, serotonin, and endothelium-dependent relaxations to platelets. Am J Physiol 1985;248:H389-95.

38. Kontos HA, Wei EP. Cerebral arteriolar dilations by KATP channel activators need L-lysine or L-arginine. Am J Physiol 1998;274:H974-81.

39. Yang J, Yu M, Jan YN, Jan LY. Stabilization of ion selectivity filter by pore loop ion pairs in an inwardly rectifying potassium channel. Proc Natl Acad Sci U S A 1997;94:1568-72.

40. Miller VM, Flavahan NA, Vanhoutte PM. Pertussis toxin reduces endothelium-dependent and independent responses to alpha2-adrenergic stimulation in systemic canine arteries and veins. J Pharmacol Exp Ther 1991;257:290-3.

41. Niu XW, Meech RW. The effect of polyamines on KATP channels in guinea-pig ventricular myocytes. J Physiol 1998;508:401-11.

42. Szabo C, Southan GJ, Thiemermann C, Vane JR. The mechanism of the inhibitory effect of polyamines on the induction of nitric oxide synthase: role of aldehyde metabolites. Br J Pharmacol 1994;113:757-66.

43. Regunathan S, Youngson C, Wang H, Reis DJ. Imidazoline receptors in vascular smooth muscle and endothelial cells. Ann N Y Acad Sci 1995;763:580-90.

44. Morrissey JJ, Klahr S. Agmatine activation of nitric oxide synthase in endothelial cells. Proc Assoc Am Physicians 1997;109:51-7.

45. Ray EC, Landis ME, Miller VM. Effects of dietary L-arginine on reactivity of canine coronary arteries. Vasc Med 1999;4:211-7.

46. Bode-Boger SM, Boger RH, Kienke S, Junker W, Frolich JC. Elevated L-arginine /dimethylarginine ratio contributes to enhanced systemic NO production in dietary L-arginine in hypercholesterolemic rabbits. Biochem Biophys Res Commun 1996;219:598-603.

47. Azuma H, Sato J, Hamasaki H, Sugimoto A, Isotani E, Obayashi S. Accumulation of endogenous inhibitors for nitric oxide synthesis and decreased content of L-arginine in regenerated endothelial cells. Br J Pharmacol 1995;115:1001-4.