

## Absence of Flow-Mediated Vasodilation in the Rabbit Femoral Artery

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### Summary

The purpose of this study was to determine if there is flow-mediated vasodilation of the femoral artery in response to progressive increases in flow within a physiological range observed in the *in vivo* experiments. Femoral artery blood flow was determined in conscious rabbits (n=5) using chronically implanted flowprobes. Resting blood flow was 8.3±0.6 ml/min and increased to 39.9±5.4 ml/min during high intensity exercise. Femoral arteries (n=12, 1705±43 µm outer diameter) harvested from a separate group of rabbits were mounted on cannulas and diameter was continuously monitored by video system. Functional integrity of the endothelium was tested with acetylcholine. The arteries were set at a transmural pressure of 100 mm Hg and precontracted with phenylephrine to 73±3 % of initial diameter. Using a roller pump with pressure held constant, the arteries were perfused intraluminally with warmed, oxygenated Krebs' solution (pH=7.4) over a physiological range of flows up to 35 ml/min. As flow increased from 5 ml/min to 35 ml/min, diameter decreased significantly (p<0.05) from 1285±58 µm to 1100±49 µm. Thus, in vessels with a functional endothelium, increasing intraluminal flow over a physiological range of flows produced constriction, not dilation. Based on these results, it seems unlikely that flow-mediated vasodilation in the rabbit femoral artery contributes to exercise hyperemia.

### Key words

Blood flow • Conduit vessel • Endothelium • Skeletal muscle

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### Introduction

Flow-mediated vasodilation is known to be elicited by changes in blood flow (Melkumyants *et al.* 1987, Rodbard 1966) which cause shear stress-induced release of nitric oxide and prostacyclin by the endothelium (Busse *et al.* 1985, Cooke *et al.* 1991, Messina *et al.* 1977, Pohl and de Wit 1999). These vasodilators diffuse to adjacent vascular smooth muscle, producing relaxation. Functional integrity of the endothelium is essential to this response because its removal can abolish the observed vasodilation (Furchgott and Zawadzki 1980, Hull 1986, Koller and Kaley 1991, Pohl 1986, Rubanyi 1986, Smieško 1985). Although resistance arterioles within the muscle are considered the primary site of regulation of blood flow through skeletal muscle, several studies have shown that feed arteries and larger conduit arteries also play an important role (Lash 1994, Mullen *et al.* 2001, Shoemaker *et al.* 1997, Sinoway *et al.* 1989, Williams and Segal 1993). For example, it has been asserted that dilation of feed arteries and conduit arteries is important for the full expression of exercise hyperemia (Segal 1992, Segal and Duling 1987). Since these vessels are not directly exposed to substances in the muscle interstitium, metabolites released by contracting skeletal muscle cannot explain their dilation. One plausible explanation is that metabolic dilation of resistance arterioles within the muscle initiates increases in blood flow through the vascular network resulting in dilation of feed arteries *via* flow-mediated mechanisms.

A complicating factor in some previous studies of flow-mediated vasomotor responses has been the use of subphysiological flows and pressures (Cooke *et al.*

1991, Sipkema *et al.* 1989, Tesfamariam and Halpern 1987). Jasperse and Laughlin (Jasperse and Laughlin 1997) made the first attempt to compare the *in vitro* responses to flow with the physiological range of flows in the same vessel. They observed vasodilation in soleus feed arteries (135  $\mu\text{m}$ ) up to flows of 14  $\mu\text{l}/\text{min}$ , but no additional dilation with increases in flow up to 65  $\mu\text{l}/\text{min}$ . Based on published data in the literature, the authors estimated that the normal physiological blood flow in rat soleus feed arteries during standing rest was 95  $\mu\text{l}/\text{min}$  (50 % more than the highest flow they employed) and during running reached 225  $\mu\text{l}/\text{min}$ . It was concluded that flow-mediated vasodilation of feed arteries probably does not contribute to exercise hyperemia. There are two limitations to this study: the normal blood flow through these arteries was estimated, rather than directly measured, and the range of flows over which diameters were measured was well below the range estimated for normal physiological blood flows.

The present study was designed to extend the findings of Jasperse and Laughlin (1997) by investigating flow-mediated responses of arteries in which blood flow could be directly measured. We examined whether there is flow-mediated vasodilation of rabbit femoral artery in response to progressive increases in flow within a physiological range observed in the *in vivo* experiments. In the first set of experiments, direct measurements of femoral artery blood flow were made at rest and during graded exercise in order to determine a physiological range of flows for the *in vitro* studies. In the second part of the study, femoral arteries were studied *in vitro* and diameters measured over the physiologically relevant range of blood flows.

## Methods

All experimental procedures performed were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the American Physiological Society's Guiding Principles in the Care and Use of Animals. Two experimental protocols were performed in order to determine physiological flows through femoral artery of the rabbit and to test flow-mediated vasodilation.

### *Protocol 1. Determination of physiological range of flows*

Five New Zealand rabbits, (mean  $3.5 \pm 0.2$  kg) selected for their willingness to run on a motorized treadmill, were surgically instrumented for measurement

of femoral artery blood flow. For surgical procedures, rabbits were initially anesthetized with 15 mg/kg Telazol (tiletamine and zolazepam, Lederle Parenterals, Puerto Rico) and 5 mg/kg Xylazine (Phoenix Scientific, St. Joseph, MO) given intramuscularly. Anesthesia was maintained by mechanical ventilation with 1.5-2.0 % isoflurane in room air. In order to measure blood flow through the femoral artery, a 1.5 mm ultrasonic transit-time flowprobe (Transonic Systems, Ithaca, NY) was placed around the femoral artery in the midsection of the thigh. The lead was then tunneled under the skin to a subcutaneous pocket made between the scapulae. Before any experiments were performed, the rabbits were given at least 10 days to recover from surgery and for air in the acoustic window of the flowprobes to be displaced by fibrous tissue.

On the day of the experiment, the skin above the subcutaneous pocket was anesthetized with local injection of lidocaine, the flowprobe connector was exteriorized and connected to a flowmeter. For continuous measurement of arterial blood pressure, a 24-gauge intravascular catheter (Insyte, Becton Dickson, Deseret, Sandy, UT) was inserted retrogradely into the lumen of the central ear artery and attached to a solid-state pressure transducer (Ohmeda, Madison, WI) secured at the level of the heart. The rabbit rested quietly for 20 min in an enclosed box while resting flows were recorded. After transfer to a motorized treadmill, the rabbit ran until volitional fatigue using a continuous protocol consisting of 3 min of exercise at the following levels: 12 m/min at 0 % grade, 15 m/min at 0 % grade, 15 m/min at 15 % grade, 17 m/min at 15 % grade, 20 m/min at 15 % grade, 22 m/min at 15 % grade, 25 m/min at 15 % grade. Systemic arterial blood pressure and blood flow signals were recorded continuously using a MacLab data acquisition system (AD Instruments, Castle Hill, Australia) sampling at 100 Hz and stored on microcomputer (Apple G3 Power PC). Blood flows were averaged over 30 s of stable recordings at rest and at the highest intensity of exercise achieved.

### *Protocol 2. Flow-mediated vasodilation*

Nine New Zealand rabbits averaging  $3.5 \pm 0.2$  kg were used in this protocol. Rabbits were anesthetized with Telazol and Xylazine (15 mg/kg and 5 mg/kg plus additional doses as needed) and the femoral vessels were harvested from the inguinal ligament to the point of femoral artery bifurcation. The vessels were stored until use in cold (4 °C) physiological saline solution (PSS)

containing (in mM) 141 Na<sup>+</sup>, 4.7 K<sup>+</sup>, 125 Cl<sup>-</sup>, 2.5 Ca<sup>2+</sup>, 0.72 Mg<sup>2+</sup>, 41.7 H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 25 HCO<sub>3</sub><sup>-</sup>, and 11 glucose.

As described previously (Shimoda *et al.* 1996), the system used to study cannulated arteries consisted of a water-jacketed plastic chamber in which proximal (inflow) and distal (outflow) cannulas are mounted. The cannulas were made of polyethylene tubing (PE 50, Intramedic, Becton Dickson, Deseret, Sandy, UT) with the tips cut so that the inflow and outflow cannula-tip diameters were equal. An arterial segment (~15 mm length oriented in the same direction as *in vivo*) was tied in place on the proximal cannula with nylon suture (size 10-0), and the lumen was flushed with PSS. The other end of the artery was tied onto the distal cannula and all side branches were ligated. The exterior of the vessel was suffused with PSS from a reservoir at 37 °C and aerated with a gas mixture containing O<sub>2</sub>, CO<sub>2</sub>, and N<sub>2</sub>, giving a PO<sub>2</sub> of 130-150 mm Hg, PCO<sub>2</sub> of 37-40 mm Hg, and pH 7.37. The artery was filled with warmed, aerated PSS and pressurized to 100 mm Hg. A micrometer connected to the proximal cannula was then used to take out the slack in the artery. In order to produce a physiologically relevant degree of tone, the vessels were precontracted with phenylephrine (0.5-1.0 × 10<sup>-5</sup> M) added to the chamber. The artery was allowed to stabilize for 60-90 min without flow before study.

Pressure and flow were independently controlled: the flow by a roller pump and the pressure by a micromanipulator servomechanism (Madden and Christman 1999). A roller pump (Cole-Parmer, Vernon Hills, IL) connected to the inflow cannula permitted the manipulation of intraluminal flows. A pulse dampener was used to minimize oscillations in flow caused by the roller pump. The pressure servocontroller consisted of an electronically driven micromanipulator (Oriol A18008 Encoder Mike, Oriol, Stratford, CT) which adjusted the resistance of the tubing connected to the outflow cannula. The motion of the micromanipulator was driven by a custom designed feedback control circuit which compared the pressures measured by the inflow and outflow pressure transducers and the pressure set by the user (Madden and Christman 1999). The transmural pressure value displayed on the controller agreed within ±1 mm Hg of the luminal pressure recorded with the micropuncture system (Shimoda *et al.* 1997). Step changes in flow caused <5 mm Hg transient changes in transmural pressure (measured on the controller and with a micropipette), which stabilized within 10±0.4 s.

A color video camera (Panasonic Digital 5000)

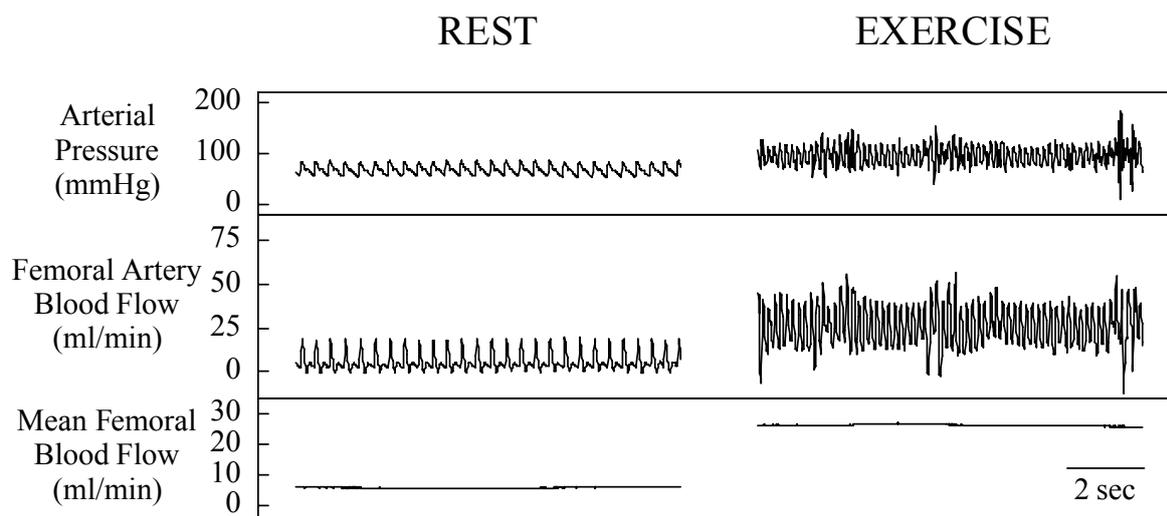
mounted on a stereomicroscope (Olympus SZ-STB1) above the vessel chamber projected the artery image on a video monitor (Sony PVM-1390), and the external arterial diameter (±1.5 μm) was measured by using a video scaler (FORA IV-550). The external diameter was always measured at the same point on the arterial wall, as judged by the presence of various distinguishing features such as adhering connective tissue or side branches located near the site. Diameters were measured after the artery was mounted, after equilibration with phenylephrine for 60-90 min, and throughout the protocols described below. Diameter values are reported as mean ± SE, expressed in μm.

Viability of the arteries was tested by measuring the contractile response to phenylephrine. The vessels were tested for a functionally intact endothelium by adding 10<sup>-6</sup> M acetylcholine at the end of the experiments. Arteries that did not contract to phenylephrine and/or dilate (>20 % change in diameter) to acetylcholine were discarded. The effects of flows from 0.6 to 35 ml/min on arterial diameter were studied, while transmural pressure was held constant at 100 mm Hg. This range of flows was chosen from results obtained in Protocol 1 and was limited by the ability to control pressure within a given range. Flow was maintained for 3 min after each step change, at which time the external diameter measurement was recorded. Flows that were employed in a continuous sequence were: 0.6 ml/min, 1 ml/min, 5 ml/min, 10 ml/min, 15 ml/min, 20 ml/min, 30 ml/min, and 35 ml/min. At the end of every experiment maximal dilation was obtained by adding 10<sup>-4</sup> M papaverine to the chamber.

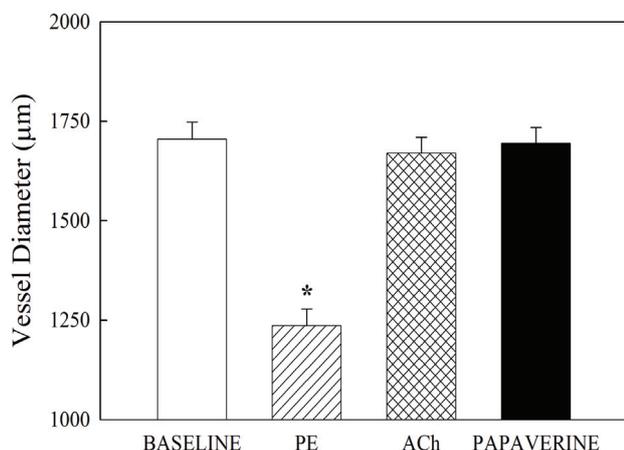
Arterial wall shear stress was calculated according to the formula:  $\tau = 4\eta \cdot Q / \pi r^3$  (Jasperse and Laughlin 1997) where  $\tau$  is shear stress (in dyne per cm<sup>2</sup>),  $\eta$  viscosity (in dyne·s/cm<sup>2</sup>),  $Q$  flow rate (in cm<sup>3</sup>/s), and  $r$  radius of the artery (in cm). Viscosity of the PSS solution was taken to be 0.008 in dyne·s/cm<sup>2</sup>. Internal radius of the femoral artery was calculated by subtracting wall thickness (LaDisa Jr *et al.* 2005; J.F. LaDisa Jr., personal communication) from the measured external diameter.

#### Statistical analysis

Changes in diameter at different flow rates and the vasomotor responses to drug administration were analyzed by one-way repeated measures analysis of variance. Where significant F-ratios were found, a Tukey's *post-hoc* test was performed. A value of P<0.05 was considered statistically significant.



**Fig. 1.** Representative example of femoral blood flow measurements in an individual rabbit at rest and at the highest level of exercise achieved. Note five-fold increase in blood flow at highest level of exercise compared to rest.



**Fig. 2.** Summary of the femoral artery diameter change in response to phenylephrine (PE), acetylcholine (ACh) and papaverine. Phenylephrine and papaverine were applied in the chamber and acetylcholine was administered intraluminally. Phenylephrine produced significant reduction in arterial diameter. Acetylcholine and papaverine dilated precontracted vessels back to original diameter at the end of experiment. \* Significantly different from baseline ( $P < 0.01$ ).

## Results

### Protocol 1

The start of treadmill exercise evoked immediate increases in blood flow through the femoral artery of the rabbit. Arterial flow was augmented as the intensity of exercise increased and reached its peak flow at the highest level of exercise. Figure 1 displays original tracings from an individual rabbit of the femoral artery blood flow and the blood pressure response at rest and at maximal exercise. For the five rabbits, femoral artery

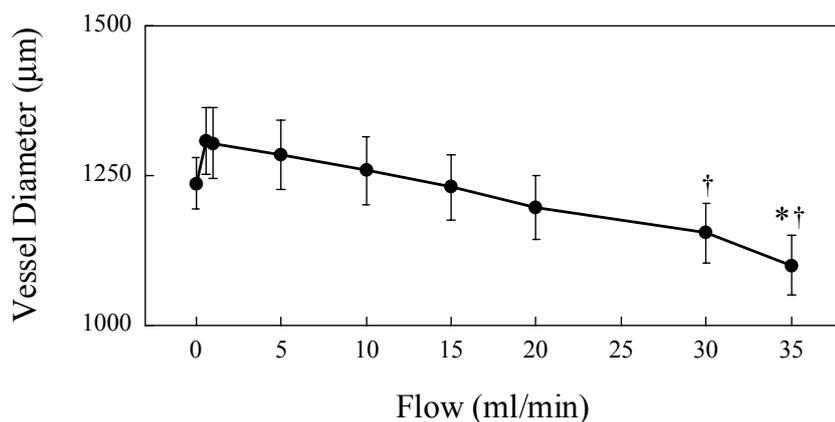
blood flow averaged  $8.3 \pm 0.6$  ml/min at rest and  $39.9 \pm 5.4$  ml/min at the highest intensity of exercise achieved.

### Protocol 2

As shown in Figure 2, arteries mounted and exposed to a pressure of 100 mm Hg had a mean diameter of  $1705 \pm 43$   $\mu\text{m}$ . After the equilibration period phenylephrine significantly ( $P < 0.01$ ) constricted arteries to  $1236 \pm 43$   $\mu\text{m}$  which represented  $73 \pm 3$  % of their initial diameter. At the end of every experiment, the functional integrity of the endothelium was verified by intraluminal administration of acetylcholine which resulted in vasodilation to  $1669 \pm 40$   $\mu\text{m}$ . The arteries were then maximally relaxed with papaverine to  $1694 \pm 40$   $\mu\text{m}$ . Neither value was significantly different from starting spontaneous diameter, indicating the absence of spontaneous tone in these vessels.

Figure 3 depicts changes in arterial diameter in response to increasing rate of flow. Initiation of flow at a rate of 0.6 ml/min produced a small but statistically insignificant increase in vessel diameter. Further increases in flow rate produced progressive constriction, which was significant at the highest flow rate compared to the no flow condition. It is worth pointing out that there were significant ( $p < 0.05$ ) reductions in arterial diameter from the 5 ml/min flow rate (just below physiological resting flow) to 30 ml/min and 35 ml/min flow rates (equivalent to moderate exercise).

Calculated values for shear stress ranged from  $1.4 \pm 0.3$  dynes/cm<sup>2</sup> at 0.6 ml/min of flow to  $193.5 \pm 40.2$  dynes/cm<sup>2</sup> at 35 ml/min of flow.



**Fig. 3.** Summary of femoral artery diameters (mean  $\pm$  SE) in response to increasing rate of intraluminal flow. Note the overall trend for constriction with increasing flow. \* Significantly different from baseline ( $P < 0.05$ ). † Significantly different from 5 ml/min (value just below resting blood flow).

## Discussion

The purpose of this study was to determine if there is flow-mediated vasodilation of rabbit femoral artery in response to progressive increases in intraluminal flow within a physiological range observed *in vivo* experiments. The data show that this skeletal muscle conduit artery did not dilate in response to increased flow or increased shear stress exerted on the walls of the vessels. On the basis of these results, it seems unlikely that flow-mediated vasodilation in the femoral artery contributes to exercise hyperemia in the rabbit.

Flow-mediated control of vessel diameter has been a subject of intense research activity over recent years. As flow rate through a vessel increases, there is a proportional increase in shear force exerted on the vessel wall (provided vessel diameter does not increase proportionately). Those forces are sensed by endothelial cells which release vasoactive substances in order to increase vessel diameter. This is a feedback mechanism which limits the elevation in shear stress during rapid changes in blood flow (Koller and Kaley 1991, Rodbard 1966). It is important to recognize that shear stress is not the sole determinant of vessel diameter. After observing that increases in shear stress failed to cause dilation in 2A, 3A, and 4A arterioles of cremaster muscle, Kurjiaka and Segal (1996) argued that the autoregulation of blood flow prevails over maintaining a constant shear stress.

The mechanisms underlying flow-mediated vasodilation have been studied in several different preparations. *In vitro* experiments permit easy manipulation of intraluminal flow while continuously monitoring vessel diameter. However, most studies have used extremely low flows which may have limited physiological relevance. *In situ* approaches leave the vessel in its natural environment within a vascular bed,

but manipulations in flow are more difficult. Studies of the cremaster vascular bed employ occlusion of branch or an arteriolar bifurcation while measuring diameter in the other branch. A recent publication provides evidence that the signal for vasodilation in this model arises from ischemia or reduced pressure distal to the site of occlusion (Dora *et al.* 2000). In humans, *in vivo* studies of conduit arteries are possible with increases in flow produced by reactive hyperemia. This requires initial reduction of flow to zero which is not a flow normally encountered under physiological conditions (Mullen *et al.* 2001, Sinoway *et al.* 1989). There is no perfect system for studying flow-mediated vasodilation and the limitations of this study should be noted. While we employed a physiological range of flows observed in conscious animals, the perfusate was PSS but not blood. Thus, although the flow rates were appropriate, the shear stress exerted on the vessel wall was less than that encountered in this vessel under *in vivo* conditions due to lower viscosity of the PSS solution compared to blood. It should be noted that PSS is the standard solution for *in vitro* perfusion of vessels in such studies (Cooke *et al.* 1991, Sipkema *et al.* 1989, Tesfamariam and Halpern 1987). Another limitation of this study is measurement of external diameter of the rabbit femoral artery, while internal diameters are required for calculation of shear stress. However, if vasoconstriction prevails (as observed in our experiments) wall thickness of the vessel increases due to constriction of vascular smooth muscle. For the calculation of shear stress we subtracted same wall thickness value for all range of flows applied. In this manner, magnitude of shear stress during highest flow is underestimated when compared to shear stress during low flows.

Our data show not only the absence of flow-mediated vasodilation, but we also observed a

progressive constriction over the range of physiological flows. Sipkema *et al.* (1989) also described flow-mediated vasoconstriction in rabbit femoral arteries. However, pressure was held at only 50 mm Hg and the maximum flow rate was 3.33 ml/min. Our results from Protocol 1 show that normal resting femoral blood flow is about 8 ml/min, so that the flows tested by Sipkema *et al.* (1989) were not in the physiological range. There are other reports of vessels which do not exhibit flow-mediated vasodilation. For example, isolated feline pulmonary (Shimoda *et al.* 1997) and cerebral (Madden and Christman 1999) arteries constrict in response to increases in perfusion rate through the vessel. Piglet cerebral arteries show constriction at lower flows and dilation at higher flows (Shimoda *et al.* 1996). Although not evaluated in this study, tyrosine kinase and integrin signaling have been implicated in the mechanism of flow-mediated constriction (Madden and Christman 1999).

The present results add to the growing body of literature examining endothelial function and blood flow. Vasodilators released from the endothelium with a potential role in flow-mediated vasodilation include nitric oxide (Ignarro *et al.* 1988, Khan and Furchgott 1987), prostacyclin (Messina *et al.* 1977, Duffy *et al.* 1998, Pohl *et al.* 1987) and endothelium-derived hyperpolarizing factor (Campbell *et al.* 1996, Miura and Guttermann 1998). The contributions of nitric oxide and prostacyclin have been studied in both animals and humans primarily by administration of competitive nitric oxide synthesis inhibitors and cyclooxygenase inhibitors, respectively. A nearly universal finding has been that inhibiting production of NO and prostaglandins reduces resting blood flow (Duffy *et al.* 1998, Gilligan *et al.* 1994, O'Leary *et al.* 1994, Wilson and Kapoor 1993a,b). It is interesting to note that pulsatile blood flow in the rabbit femoral artery approaches zero during each cardiac cycle (Fig. 1). This observation coupled with the findings of dilation in skeletal muscle vessels only at low flows (present data, Jasperse and Laughlin 1997) suggest that mechanical forces exerted on endothelial cells at low flows could account for baseline release of endothelial vasodilators.

There is not unanimity of opinion with regard to the influence of endothelial factors in exercise hyperemia. Some investigators have found no effect of NO synthase blockade on exercise blood flow (O'Leary *et al.* 1994,

Wilson and Kapoor 1993a, Brock *et al.* 1998), while others have shown a modest reduction in exercise blood flow (Dyke *et al.* 1995, Hirai *et al.* 1994). The prevailing opinion is that prostaglandins do not play a role in exercise-induced vasodilation (Wilson and Kapoor 1993b, Shoemaker *et al.* 1996). One difficulty encountered by investigators is interpretation of the magnitude of exercise hyperemia when there is a reduction in baseline blood flow. When there are parallel reductions in resting blood flow and exercise blood flow, the appropriate conclusion seems to be that there is no influence of NO on exercise hyperemia (Endo *et al.* 1994). In the present study, the role of NO or prostaglandins was not specifically addressed, but the results show that no dilation occurred in the femoral artery *in vitro* to increases in flow which match those observed during exercise *in vivo*. In the absence of flow-mediated vasodilation, what other mechanism could account for vasodilation of conduit vessels and feed arteries during dynamic exercise? The most likely candidate is conducted vasodilation, a process in which a locally induced dilation is transmitted to remote sites by direct coupling between endothelial cells and/or smooth muscle cells (Segal 2000). This principle was first established by topical application of acetylcholine to arterioles (Segal and Duling 1987), but has recently been demonstrated in response to muscle contraction (Cohen and Sarelius 2002).

In summary, the data from present experiment show that flow-mediated vasodilation is absent in the femoral artery of the rabbit over the physiological range of flows observed in that vessel. On the basis of these results, it seems unlikely that flow-mediated vasodilation in the rabbit femoral artery contributes to exercise hyperemia.

### Conflict of Interest

There is no conflict of interest.

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