

NO RELATIONSHIP BETWEEN PROGRESSIVE MUSCLE HYPERAEMIA AND TEMPERATURE IN EXERCISING RATS

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Accepted 6 July 1988

Summary

During prolonged submaximal exercise muscle blood flow has been shown to increase progressively in rats and miniature swine. This study was designed to test the hypothesis that the increases in muscle blood flow are associated with progressive elevations in body temperature in rats. Colonic temperature and muscle blood flow (determined using radioactive microspheres) were measured after 15, 30 and 45 min of exercise in rats exercising on a treadmill at 15 m min⁻¹ on a 0° incline. Total hindlimb muscle blood flow increased from 79 ± 8 ml min⁻¹ 100 g⁻¹ at 15 min to 95 ± 10 ml min⁻¹ 100 g⁻¹ at 30 min ($P < 0.05$). The greatest increases in blood flow occurred in the deep extensor muscles of the hindlimb. For example, in the red portion of the gastrocnemius muscle, blood flow increased from 197 ± 15 ml min⁻¹ 100 g⁻¹ at 15 min to 285 ± 17 ml min⁻¹ 100 g⁻¹ at 30 min ($P < 0.05$). Colonic temperature, however, remained stable at 38.5°C over this period. These data indicate that the progressive hyperaemia in muscle was unrelated to body temperature.

Introduction

During prolonged submaximal exercise, skeletal muscle blood flow has been shown to increase progressively in rats (Laughlin & Armstrong, 1983; Armstrong & Laughlin, 1985*b*) and miniature swine (Armstrong *et al.* 1987*b*). As these animals approach exhaustion during a prolonged treadmill run, muscle blood flow is almost as high as during short bouts of high-speed running (Armstrong & Laughlin, 1985*a*; Armstrong *et al.* 1987*a*). The mechanism responsible for this progressive hyperaemia during prolonged submaximal exercise is unknown. One factor that could be involved is a gradual increase in body temperature. In miniature swine performing prolonged treadmill exercise at 70% of maximal oxygen consumption, colonic temperature increases linearly at a rate of about 0.1°C min⁻¹ (McKirnan *et al.* 1986; Armstrong *et al.* 1987*b*); their inability to thermoregulate appears to limit their performance time.

Key words: blood flow, metabolism, circulation, exercise.

There are several reasons for suspecting that an increase in muscle blood flow might be related to elevations in body temperature. First, an increase in temperature increases the temperature coefficient (Q_{10}) and, hence, the metabolic rate of the skeletal muscle (Edwards *et al.* 1972; Rome & Kushmerick, 1983). Presumably this would promote the release of vasodilatory metabolites. Second, an elevation in temperature promotes the deactivation of norepinephrine (Rogers *et al.* 1965; Knight & McGregor, 1974) and/or inhibits the release of catecholamines (Lindman & Löffelholz, 1972), both of which would have the effect of decreasing sympathetic vasoconstriction in the affected tissues. Finally, an increase in temperature decreases the contractile activity of the vascular smooth muscle by (a) decreasing the sensitivity of the cell membrane to vasoconstrictor agonists (Peiper *et al.* 1971; Vanhoutte, 1980), (b) augmenting vasodilatation due to an enhanced Na^+/K^+ exchange between the smooth muscle cell and the extracellular space (Jones & Karreman, 1969; Friedman *et al.* 1973), and/or (c) increasing the efflux of sarcoplasmic Ca^{2+} to the extracellular space (Somlyo *et al.* 1971; Vanhoutte, 1980). There is therefore evidence in the literature that progressive elevations in temperature could affect progressive hyperaemia in the muscles.

Although increasing temperatures have been observed in pigs during prolonged exercise (Armstrong *et al.* 1987b), it is not known if rats experience progressive hyperthermia under the exercise conditions employed in the blood flow experiments. The first step in testing the hypothesis that the blood flow changes in the muscles are caused by increases in temperature was to determine whether temperature increases in rats during the period of increasing muscle hyperaemia.

Therefore, the purpose of the present study was to determine if increases in muscle blood flow during prolonged, low-intensity treadmill exercise in rats are associated with an increase in core temperature.

Materials and methods

Animal care and training

Seven male Sprague–Dawley rats weighing 400–575 g were used. The animals were obtained at 250–350 g, and were taught to fast-walk on a motor-driven treadmill for 2–4 weeks prior to the experiment. During this period each rat walked at 15 m min^{-1} on the treadmill for 10–15 min per day for 5 days per week. They were housed 2–4 to a cage, fed rat chow and watered *ad libitum*, and maintained at $23 \pm 2^\circ\text{C}$ with 12 h of light per day.

Experimental protocol

Following the treadmill training period described above, the rats fast-walked on the treadmill at 15 m min^{-1} for 80 min or until they could not maintain the pace. No electrical stimulation was used. Six of the rats continued for 80 min; all the animals walked for at least 45 min. Colonic temperatures were monitored continuously during the exercise bout with a Gould temperature probe and

recorder. This initial (pre-surgery) exercise bout was performed to act as a control for the potential effects of the subsequent surgery on colonic temperature in the actual experiment (see below). 4–6 days after the prolonged walk, catheters were implanted surgically for subsequent blood flow measurements with microspheres. Two days after surgery, the animals again performed prolonged treadmill walking at 15 m min^{-1} for 45–60 min. Colonic temperature, heart rate and mean arterial pressure were continuously monitored throughout the exercise, and radiolabelled microspheres were used to measure muscle blood flows after 15, 30 and 45 min of exercise. These times were chosen because previous work (Laughlin & Armstrong, 1983) indicated this period to be when the greatest increases in muscle blood flow occur during low-intensity exercise.

Surgical procedures

Catheters were implanted for blood flow measurements as described previously (Laughlin *et al.* 1982). In each rat one silastic catheter (i.d. 0.6 mm, o.d. 1.0 mm) was placed in the ascending aorta *via* the right carotid artery and another in the descending aorta *via* the left renal artery while the rats were under sodium pentobarbital anaesthesia (initial dose of 35 mg kg^{-1} , intraperitoneally). The catheters were externalized in the midscapular region, and filled with a heparinized ($500 \text{ units ml}^{-1}$) glucose (50 %) solution. The rats were allowed to recover for 2 days before the experiment.

Blood flow measurements

Procedures for blood flow measurement have been described previously (Laughlin *et al.* 1982). Radiolabelled $15 \mu\text{m}$ microspheres (New England Nuclear) tagged with ^{46}Sc , ^{57}Co , ^{85}Sr or ^{113}Sn were used. Blood flows were measured after 15, 30 and 45 min of exercise in each rat by using three separate microsphere infusions, each with a different isotope. Each infusion consisted of 5×10^5 spheres suspended in 0.1 ml of saline with 0.1 % Tween 80, followed by 1 ml of warm saline. The microspheres were delivered through the carotid catheter while a reference sample was being withdrawn at a known rate from the renal catheter. The sphere infusions occurred over about 20 s while the animal continued walking.

Following the exercise bout, the rats were anaesthetized with sodium pentobarbital and decapitated. Hindlimb muscle and other tissue samples were dissected free, weighed and counted in a Packard 5780 gamma counter, and blood flows were calculated from the counts min^{-1} and tissue sample masses (Laughlin *et al.* 1982).

Haemodynamic measurements

Heart rate and mean arterial pressure were monitored during exercise from the carotid catheter with an Ailtech pressure transducer and a Gould 2800 recorder. Systolic and diastolic pressures were not analysed because of the damping effect of the long, small-bore catheters.

Table 1. *Heart rates (HR), mean arterial pressures (\bar{P}_a), and pre- and post-surgical colonic temperatures (T_c) of seven rats during treadmill walking at 15 m min^{-1}*

Exercise time (min)	HR (beats min^{-1})	\bar{P}_a (mmHg)	Pre-surgery T_c (°C)	Post-surgery T_c (°C)
0	418 \pm 28**	114 \pm 3	37.9 \pm 0.3**	37.8 \pm 0.4**
10	439 \pm 12	107 \pm 4***	38.8 \pm 0.1*	38.4 \pm 0.3*
20	454 \pm 14	111 \pm 4	39.1 \pm 0.2*	38.5 \pm 0.3*
30	449 \pm 11	109 \pm 4**	39.1 \pm 0.2*	38.5 \pm 0.4*
40	467 \pm 24	111 \pm 4	39.1 \pm 0.2*	38.6 \pm 0.4*
50	487 \pm 36*	115 \pm 12	39.1 \pm 0.2*	38.7 \pm 1.0*

Values are means \pm S.E.M.

* Exercise value different from pre-exercise value ($P < 0.05$); ** value different from the 50 min value ($P < 0.05$).

Data analysis

A one-way analysis of variance with repeated measures was used to compare blood flows within muscles or tissues across conditions (exercise times) and to compare heart rates, blood pressures and colonic temperatures across conditions (exercise times). Duncan's new multiple-range test, in turn, was used to determine differences among treatment means. A paired *t*-test was used to compare pre- and post-surgery colonic temperatures. For all analyses the 0.05 level was chosen for statistical significance.

Results

Pre-exercise heart rate (HR) was 418 ± 28 (S.E.M.) beats min^{-1} (Table 1). There was no change in HR during exercise, except at 50 min when HR increased to 487 ± 36 beats min^{-1} . Mean arterial pressure (\bar{P}_a) decreased from 114 ± 3 mmHg (1 mmHg = 133.3 Pa) at pre-exercise to 107 ± 4 mmHg at 10 min of exercise (Table 1). At no other time did \bar{P}_a differ from that before exercise. \bar{P}_a at 50 min of exercise was 115 ± 12 mmHg, an increase above that at 10 and 30 min of exercise.

The pattern of blood flow changes in the total hindlimb musculature, as determined from the mass-average of the 32 muscles that were sampled, was for blood flow to increase from $79 \pm 8 \text{ ml min}^{-1} 100 \text{ g}^{-1}$ at 15 min to $95 \pm 10 \text{ ml min}^{-1} 100 \text{ g}^{-1}$ at 30 min of exercise (Table 2). At 45 min of exercise, blood flow declined to $86 \pm 10 \text{ ml min}^{-1} 100 \text{ g}^{-1}$. Several muscles did not show this pattern, including the white muscles [fast-twitch glycolytic (FG) fibre populations] that had uniformly low blood flow throughout the exercise period. Representative data are presented in Table 2 for the ankle extensor muscles. Individual data for all 32 muscles are not given because of their similarity to previously published values (Laughlin & Armstrong, 1983). The decline in muscle blood flows between 30 and 45 min may be attributed to the fact that most of the rats were beginning to lose pace by 45 min into the exercise bout. Thus, the period in which the greatest increases in muscle blood flow occurred was from 15 to 30 min of exercise.

Table 2. *Blood flows ($\text{ml min}^{-1} 100 \text{ g}^{-1}$) in the total hindlimb and ankle extensor muscles of seven rats during treadmill walking at 15 m min^{-1}*

Time (min)	T _H	S	P	G _R	G _M	G _W
PE†	30 ± 6	111 ± 7	17 ± 5	60 ± 14	23 ± 5	12 ± 2
15	79 ± 8	238 ± 33	97 ± 12	197 ± 15	64 ± 7	10 ± 2
30	95 ± 10*	305 ± 37*	122 ± 15	285 ± 17*	84 ± 9*	11 ± 1
45	86 ± 10	274 ± 45	107 ± 13	272 ± 36	74 ± 8	12 ± 4

Values are means ± S.E.M.

Muscles are: total hindlimb (T_H); soleus (S); plantaris (P); and the red (G_R), middle (G_M) and white (G_W) portions of gastrocnemius muscle (lateral and medial heads combined).

* Value different from the 15 min value ($P < 0.05$).

† Pre-exercise values are from Laughlin & Armstrong (1984).

Colonic temperature (T_c) during the pre-surgical trials was the same at all times as during the post-surgical (experimental) exercise bouts (Table 1). These data indicate that surgery for catheter implantation did not affect the animals' ability to thermoregulate in a normal manner during the prolonged exercise bout. T_c at all exercise times was higher than pre-exercise T_c and did not change during exercise (Table 1).

Discussion

Muscle blood flow increased between 15 and 30 min in the hindlimb of rats performing prolonged low-intensity treadmill exercise at 15 m min^{-1} . Colonic temperature remained stable during this period, indicating that the progressive elevations in muscle blood flow are not related to temperature. These conclusions are based on statistical analysis of the data. The results in Table 1 demonstrate, however, that absolute mean colonic temperature did increase by 0.1°C between 10 and 30 min of exercise, the period in which muscle blood flow increased. Nonetheless, comparison of colonic temperatures and muscle blood flows for individuals argues against a relationship between the two variables. For example, for rat number 2, colonic temperature between 10 and 30 min of exercise was constant at 39.3°C , while blood flow in the red portion of gastrocnemius muscle increased by 72%. It is possible that colonic temperature did not reflect local temperature changes in the active muscle, but this is doubtful in view of the length of the exercise period and the magnitude of the perfusion of the red muscles. The data indicate that changes in blood flow to the colon during exercise did not affect the colonic temperature recordings, since there was no relationship between blood flow and colonic temperature among animals over the three exercise times ($r = -0.07$).

We have observed the progressive increases in muscle blood flow in laboratory rats in three separate studies (present study; Laughlin & Armstrong, 1983;

Armstrong & Laughlin, 1985b) and in miniature swine (Armstrong *et al.* 1987b). In rats the increased flow with time has been measured both with microspheres (present study; Laughlin & Armstrong, 1983) and with electromagnetic flow probes (Armstrong & Laughlin, 1985b). The mechanisms that underlie the increases in muscle blood flow with time during prolonged exercise in rats and pigs are not known. Several hypotheses were suggested in a previous paper (Laughlin & Armstrong, 1983): (a) the increases in blood flow are related to increases in temperature; (b) the elevations are related to alterations in fibre recruitment that occur as the initially activated motor units fatigue; (c) the increases are related to progressive diminution of sympathetic tone in the muscle vascular beds; and (d) the changes result from accumulation of a vasodilator substance in the muscles. This study was specifically designed to shed light on the first hypothesis.

In both humans (Johnson & Rowell, 1975; Rowell, 1983) and animals (McKirnan *et al.* 1986; Armstrong *et al.* 1987b), core temperatures may increase with time during prolonged submaximal exercise, even when the exercise is performed in a thermoneutral environment. In miniature swine performing treadmill exercise at 70% $\dot{V}_{O_{2,max}}$, in which muscle blood flows progressively increase in a manner similar to those in rats, colonic temperature increases at a rate of approx. $0.1^{\circ}\text{C min}^{-1}$ (McKirnan *et al.* 1986; Armstrong *et al.* 1987b); their inability to thermoregulate appears to limit their performance time (McKirnan *et al.* 1986). The data for the pigs supported the idea that the changes in muscle flow may be related to temperature.

A continuous rise of temperature in rats performing low- to moderate-intensity steady-state treadmill exercise has not been observed by others (Gollnick & Ianuzzo, 1968). However, it seemed important to study the temperature response under the same conditions used in the blood flow studies (i.e. surgical intervention, blood withdrawal, microsphere infusions, etc.).

As noted in the previous study (Laughlin & Armstrong, 1983), when the rats began to falter on the treadmill the muscle blood flows decreased below the levels attained earlier in the exercise. In the present experiments, most of the animals began to lose pace between 30 and 45 min. No electrical stimulation was used to encourage the animals to exercise, or much longer performance times would undoubtedly have been observed. At 15 min^{-1} the rats were exercising at less than 50% of $\dot{V}_{O_{2,max}}$ (Armstrong *et al.* 1983).

The data also provide information concerning the third hypothesis proposed by Laughlin & Armstrong (1983), that changes in sympathetic tone with time explain the progressive hyperaemia. In white muscles (e.g. G_W in Table 2) and other tissues (e.g. diaphragm, gracilis muscle, colon, inguinal fat in Table 3) there were no changes in blood flow or calculated resistance between 15 and 30 min of exercise. Presumably an attenuation of general sympathetic vasoconstrictor tone in the muscle vascular beds would have resulted in decreased resistance in the inactive white muscles and other tissues. Thus, although this hypothesis (i.e. that decreased sympathetic vasoconstriction underlies the elevations in muscle blood flow) was not tested directly in the muscles that demonstrate the progressive

Table 3. Blood flows (BF) and estimated resistance (R) in various tissues of seven rats during prolonged treadmill exercise at 15 m min⁻¹

Time (min)	Diaphragm		Gracilis		Colon		Duodenum		Kidney		Inguinal fat	
	BF	R	BF	R	BF	R	BF	R	BF	R	BF	R
PE†	89 ± 16		16 ± 5		110 ± 16		240 ± 31		718 ± 54		18 ± 3	
15	89 ± 19	1.20	26 ± 5	4.12	135 ± 20	0.79	390 ± 66	0.27	726 ± 85	0.15	53 ± 4	2.02
30	71 ± 9	1.54	17 ± 2	6.41	122 ± 26	0.89	436 ± 91	0.25	760 ± 131	0.14	52 ± 7	2.10
45	71 ± 15	1.56	15 ± 3*	7.40	100 ± 16	1.11	351 ± 78	0.32	579 ± 85	0.19	34 ± 4***	3.26

The estimated resistances in the tissues were calculated from the mean blood flows and average mean arterial pressures; values are in peripheral resistance units per 100 g (mmHg ml⁻¹ min⁻¹ 100 g⁻¹).

Blood flow values are means ± S.E.M. in ml min⁻¹ 100 g⁻¹.

* Value different from the 15 min value ($P < 0.05$); ** value different from the 30 min value ($P < 0.05$).

† Pre-exercise (PE) values are from Armstrong & Laughlin (1984).

hyperaemia, these observations indicate that change in general sympathetic tone was not the causative factor.

In conclusion, this experiment was designed to shed light on the relationship between colonic temperature and the progressive rise in muscle blood flow that occurs with time during prolonged submaximal treadmill exercise in rats. The data indicate that the increases in flow are not related to a rise in core temperature. Also, the findings provide indirect evidence that the blood flow changes do not result from a gradual decrease in general sympathetic tone. The mechanism responsible for the progressive elevation in muscle blood flow with time during submaximal locomotory exercise in rats remains unknown.

We thank Phillip Delp, Deborah Jerrod and Ken Rouk for their technical help and Pamela Clark for her help in manuscript preparation. Supported by NIH grant no. AM37098. MHL is the recipient of NIH Research Career Development Award no. HL-01774.

References

- ARMSTRONG, R. B., DELP, M. D., GOLJAN, E. F. & LAUGHLIN, M. H. (1987a). Distribution of blood flow in muscles of miniature swine during exercise. *J. appl. Physiol.* **62**, 1285–1298.
- ARMSTRONG, R. B., DELP, M. D., GOLJAN, E. F. & LAUGHLIN, M. H. (1987b). Progressive elevations in muscle blood flow during prolonged exercise in swine. *J. appl. Physiol.* **63**, 285–291.
- ARMSTRONG, R. B. & LAUGHLIN, M. H. (1984). Exercise blood flow patterns within and among rat muscles after training. *Am. J. Physiol.* **246**, H59–H68.
- ARMSTRONG, R. B. & LAUGHLIN, M. H. (1985a). Rat muscle blood flows during high-speed locomotion. *J. appl. Physiol.* **59**, 1322–1328.
- ARMSTRONG, R. B. & LAUGHLIN, M. H. (1985b). Metabolic indicators of fibre recruitment in mammalian muscles during locomotion. *J. exp. Biol.* **115**, 201–213.
- ARMSTRONG, R. B., LAUGHLIN, M. H., ROME, L. & TAYLOR, C. R. (1983). Metabolism of rats running up and down an incline. *J. appl. Physiol.* **55**, R518–R521.
- EDWARDS, R. H. T., HARRIS, R. C., HULTMAN, E., KAUSER, L., KOH, D. & NORDEJÖ, L.-O. (1972). Effects of temperature on muscle energy metabolism and endurance during successive isometric contractions, sustained to fatigue, of the quadriceps muscle in man. *J. Physiol., Lond.* **220**, 335–352.
- FRIEDMAN, S. M., NAKASHIMA, M., PALATY, V. & WALTERS, B. K. (1973). Vascular resistance and Na^+ - K^+ gradients in the perfused rat tail artery. *Can. J. Physiol. Pharmacol.* **51**, 410–417.
- GOLLNICK, P. D. & IANUZZO, C. D. (1968). Colonic temperature response of rats during exercise. *J. appl. Physiol.* **24**, 747–750.
- JOHNSON, J. M. & ROWELL, L. B. (1975). Forearm skin and muscle vascular response to prolonged leg exercise in man. *J. appl. Physiol.* **39**, 920–924.
- JONES, A. W. & KARREMAN, G. (1969). Ion exchange properties of canine carotid artery. *Biophys. J.* **9**, 884–909.
- KNIGHT, A. & MCGREGOR, D. D. (1974). Development of vascular reactivity in chickens: response of mesenteric and hindlimb blood vessels to norepinephrine and acetylcholine. *Blood Vessels* **11**, 212–228.
- LAUGHLIN, M. H. & ARMSTRONG, R. B. (1983). Rat muscle blood flows as a function of time during prolonged slow treadmill exercise. *Am. J. Physiol.* **244**, H814–H824.
- LAUGHLIN, M. H., ARMSTRONG, R. B., WHITE, J. & ROUK, K. (1982). A method for using microsphere to measure muscle blood flow in exercising rats. *J. appl. Physiol.* **52**, 1629–1635.
- LINDMAN, R. & LÖFFELHOLZ, K. (1972). Differential effects of hypothermia on neuronal efflux, release and uptake of noradrenaline. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol.* **274**, 410–414.

- MCKIRNAN, M. D., GRAY, C. G., GIAMELA, R. J., GUONS, M. J. & BLOOR, C. M. (1986). Skin wetting (SW) reduces core temperature (T) in exercising swine. *Physiologist* **29**, 115.
- PEIPER, U., GRIEBEL, L. & WENDE, W. (1971). Unterschiedliche Temperaturabhängigkeit der Gefäßmuskelkontraktion nach Aktivierung durch Kalium-Depolarisation bzw. Noradrenalin. *Pflügers Arch. ges. Physiol.* **324**, 67–78.
- ROGERS, L. A., ATKINSON, R. A. & LONG, J. P. (1965). Responses of isolated perfused arteries to catecholamines and nerve stimulation. *Am. J. Physiol.* **209**, 376–382.
- ROME, L. C. & KUSHMERICK, M. J. (1983). Energetics of isometric contractions as a function of muscle temperature. *Am. J. Physiol.* **244**, C100–C109.
- ROWELL, L. B. (1983). Cardiovascular adjustments to thermal stress. In *Handbook of Physiology, The Cardiovascular System*, II, section 2, vol. 3, chapter 27 (ed. J. T. Shepherd & F. M. Abboud), pp. 967–1023. Bethesda, MD: Am. Physiol. Soc.
- SOMLYO, A. P., DEVINE, C. E., SOMLYO, A. V. & NORTH, S. R. (1971). Sarcoplasmic reticulum and the temperature-dependent contraction of smooth muscle in calcium-free solutions. *J. Cell Biol.* **51**, 722–741.
- VANHOUTTE, P. M. (1980). Physical factors of regulation. In *Handbook of Physiology, The Cardiovascular System*, section 2, vol. II, chapter 16 (ed. D. F. Bohr, A. P. Somlyo & H. V. Sparks), pp. 443–474. Bethesda, MD: Am. Physiol. Soc.