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Exercise-induced sympathetic dilatation in arterioles of the guinea pig tibial periosteum

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ABSTRACT

Strength training induces not only muscle growth but also increased bone strength, a change that is expected to be associated with increased bone blood flow. However, the effects of exercise on contractile properties of bone microvascultaure have not been investigated. Once-a-week strength training with electrical muscle stimulation was applied unilaterally to tibialis anterior muscle of guinea pigs, while muscle force was measured from both legs to compare their muscle strength and endurance. After 10 weeks of training, changes in the arteriolar diameters of isolated periosteum taken from both trained and non-trained legs were measured using a video tracking system. Electrical field stimulation evoked a phasic constriction followed by a sustained dilatation in periosteal arterioles of trained legs, while triggering only vasoconstriction in the arterioles of non-trained legs. In trained leg arterioles, phentolamine, an α-adrenoceptor antagonist, inhibited both the constriction and dilatation. Prazosin, an α_1-adrenoceptor antagonist, inhibited only the constriction, while yohimbine, α_2-adrenoceptor antagonist, or L-nitro arginine (L-NA), a nitric oxide (NO) synthase inhibitor, inhibited the dilatation. In non-trained leg arterioles, phentolamine or prazosin largely suppressed the constriction, but failed to unmask any dilatation. Consistently, noradrenaline (NAd)-induced arteriolar constriction was enhanced and prolonged by L-NA in trained but not non-trained side arterioles. Thus, NAd released from sympathetic nerves appears to activate endothelial α_2-adrenoceptors to release NO resulting in the sustained dilatation of periosteum arterioles from trained leg. The altered sympathetic vasomotor function would facilitate the blood supply to the bone and may contribute to the exercise-induced bone strength gain.

1. Introduction

Bone vasculature supply oxygen and nutrients to meet the bone metabolic demands, and thus plays fundamental roles in maintaining bone growth, remodeling and healing, while vascular dysfunction is inevitably associated with bone loss (Alagiakrishnan et al., 2003; Marenzana and Arnett, 2013). In healthy individuals or animals, bone circulation is predominately supplied by nutrient arteries that create centrifugal (i.e. outward) blood flow (Bridgeman and Brookes, 1996), while the periosteal arteries also supply the outer third of the cortical bone (Tomlinson and Silva, 2013). A major trigger of periosteal vascular insufficiency is the microvascular trauma, which subsequently causes ischemia, inflammation and nutritive dysfunction. The pathogenetic influence of trauma-induced cellular and microvascular change in periosteum is well documented in clinical observations (Kowalski et al., 1996; Utvåg et al., 1998). The vascular supply to bone tends to decline with aging, and resulting vascular dysfunction is considered to contribute to the pathogenesis of osteoporosis. In addition to the global reduction in bone blood supply in senescence, it was also demonstrated that aged bone cortex is predominately supplied from periosteal arteries rather than nutrient arteries. Consistently, investigations into the early stage degradation of bone metabolism in osteoporosis demonstrated that the periosteum blood vessels play a critical role in the induction of vascular insufficiency of bone (Fan et al., 2010). Thus, the increased importance of periosteal blood supply in aging has been highlighted.

During growth, physical activity is required to increase bone mineral density (Jendzjowsky et al., 2014), and regular exercise improves bone strength (Iwamoto et al., 2005; Modlesky and Lewis, 2002). In addition, the onset or progression of age-related decreases in bone mineral density can be delayed by regular exercise enhancing bone

Abbreviations: EFS, electrical field stimulation; EMS, electrical muscle stimulation; TH, tyrosine hydroxylase; L-NA, L-nitro arginine; NAd, noradrenaline; NO, nitric oxide; NOS, nitric oxide synthase; α-SMA, α-smooth muscle actin

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accrual during the period of bone growth and maintenance (Modlesky and Lewis, 2002). Exercise also increases muscle strength that is strongly correlated with increased bone strength (Layne and Nelson, 1999).

Change in morphological and oxidative properties are apparent in muscles after short duration contractions with electrical muscle stimulation (EMS), most notably in the transformation of fast fibres into slow fibres, increasing the muscle endurance during sustained exercise (Dudley-Javoroski, 2008). Slow fibres have a higher energetic efficiency than fast fibres, enabling the muscle to contract and maintain the force for a longer period. When a muscle is exposed to EMS, muscular metabolic activity, namely an enhancement in oxidative capacity and endurance property, is increased, resulting in muscle fatigue resistance (Martin et al., 1992).

It is well established that strength training enhances endothelial functions to release vasodilators, i.e., NO and prostaglandins associated with a decreased sensitivity to the vasoconstrictor effects of NAd (Delp, 1995; Donato et al., 2007; Koller et al., 1995; Laughlin et al., 2004; Sun et al., 1994), resulting in a reduction of vascular resistance (Proctor et al., 2001). However, there seems to be a variation in such exercise-induced vascular adaptations depending on the limb or vascular segment investigated (Jasperse and Laughlin, 2006). It has been reported that lumbar sympathetic chain stimulation-induced increase in blood flow in resting skeletal muscle was augmented following exercise training (Jendzjowsky and DeLorey, 2012). Thus, it was envisaged that strength training may upregulate sympathetic vasodilatation or diminish sympathetic vasoconstriction facilitating an increase of blood supply to the contracting muscles. Nitric oxide appears to be involved in the attenuation of vasoconstriction during sympathetic nerve stimulation to increase blood flow in skeletal muscle (Ohyanagi et al., 1992; Tesfamariam et al., 1987; Thomas and Victor, 1998).

Since the blood vessels in the periosteum are connected with the vascular bed in adjacent skeletal muscles, the enhancement in nutritional blood flow to skeletal muscle during the strength training would also increase periosteal microvascular perfusion. We have recently reported that periosteal arterioles of the guinea-pig tibia exhibit rhythmic spontaneous constrictions, namely vasomotion (Fukuta et al., 2017). Vasomotion is considered to reduce the effective vascular resistance expressed as the harmonic mean but not the time average of the instantaneous resistance. (Funk et al., 1983; Meyer et al., 2002). Periosteal arterioles are constricted by sympathetic nerve stimulation, while primary afferent nerve/endothelial nitric oxide (NO) appear to oppose sympathetic vasoconstrictions (Fukuta et al., 2017). Here, we aimed to investigate the effects of forced training of tibialis anterior muscle on the contractile properties of periosteal arterioles in the tibia, particularly focusing on the sympathetic nerve- and endothelial NO-mediated modulation of the vascular contractility.

2. Methods

2.1. Ethical approval

The experimental protocol of the present study was approved by the Nagoya City University Medical School experimental animal committee.

2.2. Electrical stimulation to the tibialis anterior muscle

Male guinea-pigs (Japan SLC, Shizuoka, Japan) were housed in plastic cages in a special temperature-controlled room (23.5 ± 2 °C, 50 ± 10% humidity) on a 12/12 h light/dark cycle with free access to food and water. Starting when they were 6 weeks old, the guinea pigs had unilateral muscle strength training with EMS performed once a week for ten weeks; animals were anesthetized by isoflurane inhalation, and a pair of surface electrodes connected to an electrical stimulator (SEN-3301 Nihon Kohden, Tokyo, Japan) were attached to left tibialis anterior muscle to electrically stimulate the muscle percutaneously. One electrode was adhered on the motor point of the tibialis anterior muscle, and the other was positioned on the proximal musculotendinous junction. Positive square pulses of 1 ms width were delivered at the frequency of 5 Hz for 400 ms, and were repeated every 5 s for 30 min. Voltage was maintained at the values of 30–40 V that were 2–3 times greater than the minimal voltage required to produce the peak twitch tension.

A force transducer (TB-611T, Nihon Kohden, Tokyo, Japan) was connected by a thread to the toe side of a plastic lamina adhered to the planta pedis with a plastic adhesive tape. The toe was continuously pulled in the direction of plantarflexion with a weight of 10 g, and the dorsiflexion force produced by the tibialis anterior muscle contraction in response to EMS was recorded.

2.3. Tissue preparation on periosteal arterioles

After 10 weeks of strength training, the guinea-pigs were anesthetized with isoflurane inhalation and exsanguinated by decapitation. Both ‘trained’ lower left leg and ‘non-trained’ lower right leg were cut off at the knee joint using dissecting scissors and immersed in physiological salt solution (PSS) for video tracking or in phosphate saline (PBS) at 4 °C for immunohistochemistry. The periosteum sheets containing microvasculature were dissected away from the tibia using sharp tweezers under a dissection microscope.

2.4. Video tracking with Dimatrak

A periosteum preparation was pinned out on a Sylgard plate at the bottom of recording chamber (volume, approximately 2 ml), which was mounted on the stage of an inverted microscope attached with a video camera. Preparations were superfused with oxygenated (95% O_2–5% CO_2) warmed (35 °C) PSS at a constant flow rate (2 ml·min^{-1}) during all functional experiments. Changes in the diameter of periosteal arterioles were monitored with a video camera and analysed using Diamtrak, a in real-time edge-tracking software (Neild, 1989).

Neural selectivity of electrical field stimulation (EFS; 100 μs duration, 10 Hz, 10 s) was confirmed by its sensitivity to 1 μM tetrodotoxin. The preparations were pretreated with phentolamine, prazosin, yohimbine or propranolol for 10 min, or L-NA for 30 min before applying EFS to ensure the blockade of target receptors or enzymes.

2.5. Immunohistochemistry

Both the ‘trained’ lower left leg and the ‘non-trained’ lower right leg of guinea pigs were dissected in phosphate buffered saline (PBS) at 4 °C. The tibialis anterior muscle was cut transversely and divided into small specimens about 2 mm thick. The periostea were fixed using Zamboni fixative or acetone as previously described (Fukuta et al., 2017), while the small specimens of tibialis anterior muscle were fixed in Zamboni fixative.

Connective tissue around the periosteum microvasculature was dissected free after fixation. The fixed specimens of tibialis anterior muscle were immersed in PBS containing 30% sucrose for a few hours, immersed in OCT compound and frozen at −80 °C. Subsequently, sections (10 μm thick) of the tibialis anterior muscle were cut using a cryostat, mounted on MAS-coated glass slides and dried.

The protocols of immunostaining were described previously (Fukuta et al., 2017). Briefly, the whole mount of periosteum and sections of tibialis anterior muscle were incubated with 0.3% Triton X-100 in PBS and then with Block Ace. Tissue was incubated with one or two primary antibodies for 4 days at 4 °C. Tissue was incubated with biotinylated swine anti-rabbit IgG antibody (1:300, Dako) for 30 min only when the rabbit primary antibody was used. Then tissue was incubated with Cy3-conjugated goat anti-mouse IgG antibody (2.5 μg/ml, Chemicon) and/or Alexa488-conjugated streptavidin (10 μg/ml, Molecular Probes) for

2 h. Specimens were observed using a confocal laser scanning microscope (LSM 5 PASCAL, Zeiss).

The primary antibodies used were mouse monoclonal antibody for fast skeletal muscle myosin (1:500, clone MY-32, Sigma), mouse monoclonal antibody for slow skeletal muscle myosin (1:4000, clone NOQ7.5.4D, Sigma), mouse monoclonal antibody for α-smooth muscle actin (α-SMA; 1:200, clone 1A4, Sigma), rabbit antibody for tyrosine hydroxylase (TH; 1:1000, Millipore) and mouse monoclonal antibody for endothelial nitric oxide synthase (eNOS, 1:500, BD Transduction Laboratories).

The percentage of slow and fast skeletal muscle myosin-immunoreactive fibres in micrographs of muscle cross section were evaluated in the different cross sections of tibialis anterior muscle. The total number of skeletal muscle fibres in micrographs were counted using the differential interference contrast images.

2.6. Solutions

The compositions of PSS was 137.5 mM Na^+, 5.9 mM K^+, 2.6 mM Ca^{2+}, 1.2 mM Mg^{2+}, 15.5 mM HCO_3^-, 1.2 mM $H_2PO_4^-$, 134.4 mM Cl^- and 11.5 mM glucose. Noradrenaline (NAd), phentolamine (α-adrenoceptor antagonist), prazosin (α_1-adrenoceptor antagonist), yohimbine (α_2-adrenoceptor antagonist), propranolol (β-adrenoceptor antagonist), L-nitro arginine (nitric oxide synthase inhibitor) were purchased from Sigma. The drugs were prepared as concentrated stock solutions in deionised water and diluted in the PSS to the required concentration. The final concentration of the solvent in the PSS did not exceed 1: 1000. The concentration of drugs used was determined based on the previous studies of microvasculature (Hashitani et al., 2012; Morris, 1994).

2.7. Data analysis

Data are expressed as mean ± SEM. The number of animals used in each experiment was shown as *n* value. Paired Student's *t*-test was used to examine the effects of drugs. Unpaired Student's *t*-test was used to make comparisons between the vessels from trained and untrained hindlimbs. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of exercise on the contractile properties of guinea-pig tibialis anterior muscle

The maximum value of the tetanic contractions in the tibialis anterior muscle during the initial 5 min period were 39.7 ± 5.6 g on the first training day (Week 1) and 56.4 ± 7.4 g ($P < 0.05$, $n = 19$, significantly different from first training day) after 10 weeks of strength training (Week 10), indicating the increased strength of the trained muscle. On the first training day, repetitive EMS-induced tetanic contractions rapidly declined during the initial 5 min period (Fig. 1a), and the mean contraction amplitude in the final 5 min period was decreased to 25.5 ± 3.3% of the initial value (Fig. 1g). After 10 weeks of strength training, EMS-induced muscle contractions were more gradually reduced during the initial 5 min period, and well maintained over the 30 min EMS at 58.1 ± 4.4% of the initial value (Fig. 1d). The ratio of mean contraction amplitude during the final and initial 5 min period was successively increased during the series of training, and reached to 58.1 ± 4.4% ($P < 0.05$, significantly different from first training day) after 10 weeks of strength training (Fig. 1g), indicating the increased endurance of trained muscle.

3.2. Muscle fibres types revealed by immunohistochemistry

After 10 weeks of strength training, immunohistochemistry for fast or slow skeletal muscle myosin revealed that tibialis anterior muscle fibres from both trained and untrained limbs were predominately immunoreactive for fast type myosin (Fig. 1d; 96 ± 1% in non-trained legs, 95 ± 1% and trained legs, $p > 0.05$, $n = 9$), while the fibres positive for slow type myosin were scarce (Fig. 1e; 5 ± 1% in non-trained legs, 7 ± 1% in trained legs, $p > 0.05$, $n = 9$). Thus, despite the increased endurance and strength of trained muscle, a corresponding phenotype change in skeletal muscle myosin isoform was not evident after 10 weeks of strength training.

3.3. Morphological characteristics of periosteal arterioles of guinea-pigs

In whole mounts of the periosteum of guinea-pig tibia, immunostaining for α-smooth muscle actin (α-SMA, vascular smooth muscle maker) revealed that second branch arterioles and venules run in parallel in both non-trained and trained legs (Fig. 2a, c). Consistent with our pervious report (Fukuta et al., 2017), the arterioles were surrounded by tightly packed circumferentially orientated smooth muscle cells, while the venules were wrapped by a sparser network of stellate shaped smooth muscle cells. The sympathetic innervation of periosteal microvessels was examined by double immunostaining for α-SMA and tyrosine hydroxylase (TH, sympathetic nerve marker). Sympathetic nerves densely projected to the periosteal arterioles in both non-trained (Fig. 2b) and trained (Fig. 2d) legs, while sympathetic innervation to the periosteal venules was not evident.

3.4. Spontaneous phasic constrictions in periosteal arterioles of guinea-pigs

In non-trained legs, second branch periosteal arterioles had a mean diameter of 23.4 ± 1.7 μm ($n = 15$). 8 out of 15 arterioles exhibited spontaneous phasic constrictions at a mean frequency of 1.7 ± 0.4 min^{-1}. Spontaneous constrictions produced a mean reduction in the resting diameter of 12.6 ± 3.5% ($n = 8$) and a mean half duration of 2.8 ± 0.5 s (n = 8) (see Fig. 3a, b, g, k). Spontaneous arteriolar constrictions were not prevented by tetrodotoxin (1 μM, $n = 3$), indicating that they are myogenic in origin.

In trained legs, second branch periosteal arterioles had a diameter of 21.9 ± 2.1 μm ($n = 15$) and 10 out of 15 arterioles exhibited rhythmic spontaneous constrictions. Neither of these measures differed from those of non-trained legs. However, spontaneous constrictions were generated at a higher mean frequency of 2.9 ± 0.5 min^{-1} ($n = 10$) compared with non-trained leg ($P < 0.05$). Spontaneous constrictions produced a mean reduction in the resting diameter of 17.8 ± 3.0% (n = 10) and mean half duration was 4.0 ± 1.0 s (n = 10, see Fig. 4a,d,g,j). These values were not significantly different from those of corresponding parameters in the arterioles of non-trained leg.

3.5. Nerve-evoked constriction in periosteal arterioles of non-trained leg

In periosteal arterioles of non-trained legs, electrical field stimulation (EFS, 100 μs duration, 10 Hz for 10 s) evoked a phasic constriction ($n = 12$, Fig. 3a,d,g,j). EFS-evoked vasoconstriction was attenuated by phentolamine (1 μM), a non-selective α-adrenoceptors antagonist ($n = 7$, Fig. 3b, c). Blockade of α_1-adrenoceptors with prazosin (1 μM) also greatly attenuated these nerve-evoked vasoconstrictions ($n = 6$, Fig. 3e, f). Blockade of α_2-adrenoceptors with yohimbine (1 μM, $n = 5$, Fig. 3h, i) or β-adrenoceptors with propranolol (1 μM, n = 6, Fig. 3k, l) did not attenuate the nerve-evoked constrictions.

3.6. Nerve-evoked responses in periosteal arterioles of trained leg

In periosteal arterioles of trained legs, EFS (100 μs duration, 10 Hz for 10 s) evoked phasic constriction ($n = 10$, Fig. 4a,d,g,j) followed by a sustained dilatation ($n = 10$, Fig. 4a,d,g,j). Phentolamine attenuated both the EFS-evoked vasoconstriction ($n = 10$, Fig. 4b, c) and vasodilatation ($n = 10$, Fig. 4b, c). Prazosin attenuated EFS-evoked vasoconstriction (n = 5, Fig. 4e, f) but not vasodilatation ($n = 5$, Fig. 4e, f). Yohimbine attenuated EFS-induced vasodilatation ($n = 4$, Fig. 4h, i) but

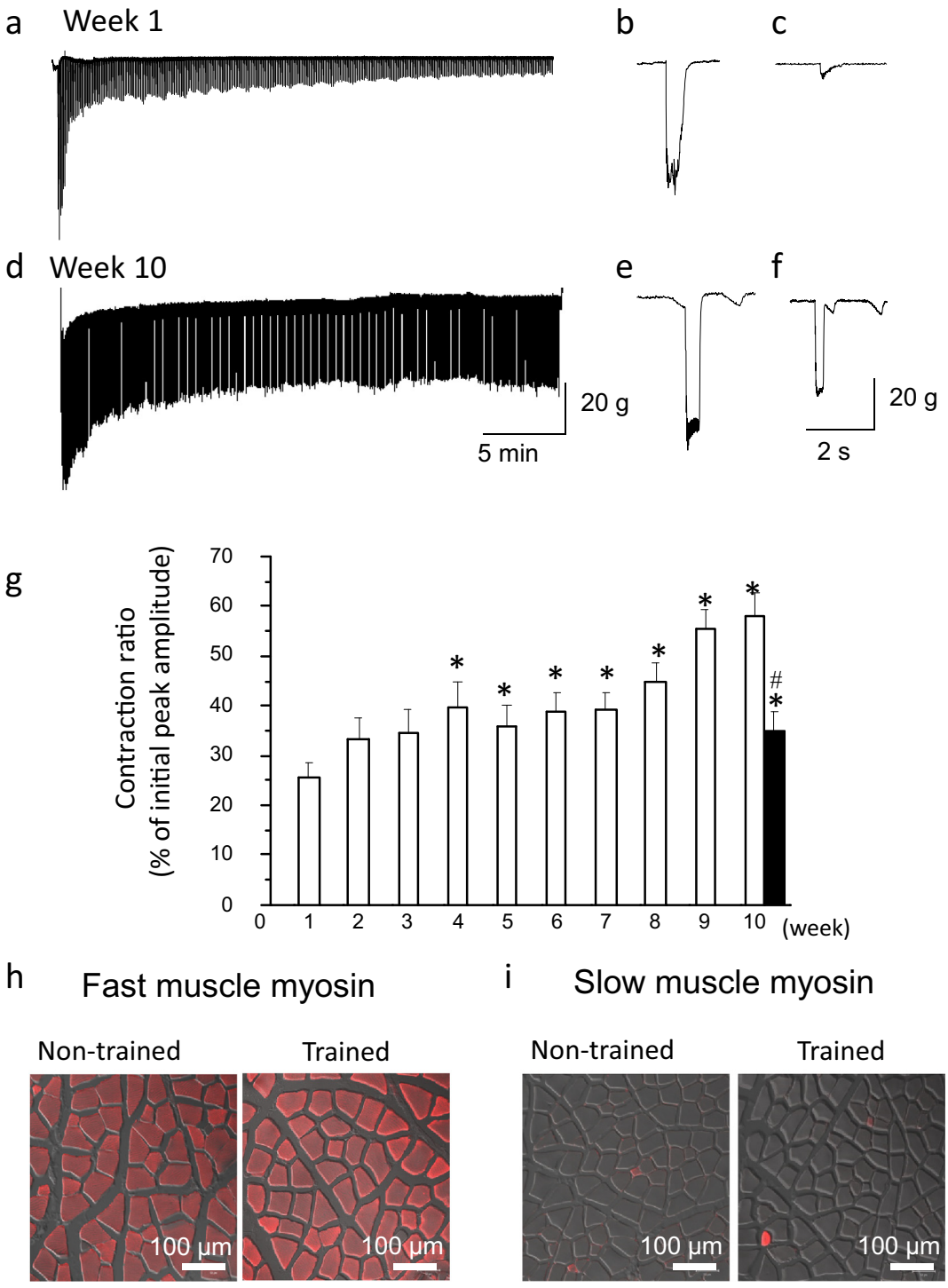

Fig. 1. Effects of 10 week training session on the contractile and morphological properties in tibialis anterior muscle of the guinea-pig
Changes in the contractile force of guinea pig tibialis anterior muscle during the whole period of electrical muscle stimulation (EMS, 5 Hz for 400 ms, repeated every 5 s for 30 min) in Week 1 (a) and Week 10 (d). At Week 1 and Week 10, representative tetanic muscle contractions are displayed on an expanded time scale during the initial (b and e, respectively) and final (c and f, respectively) 5 min period. These results were summarized in (g) demonstrating successive changes in the ratio of the final 5 min period/the initial 5 min period (%) of mean contractions in trained leg during the 10 weeks session of training (open bar). In Week 10, the ratio of mean contraction amplitude in trained leg was significantly higher than that in non-trained leg (closed bar) ($n = 5$, $\#P < 0.05$). In Week 4 and thereafter, the ratio of mean contraction amplitude in trained leg was higher than that of Week1 ($n = 19$, $^*P < 0.05$). In Week 10, most of muscle fibres in the cross sections of tibialis anterior muscle expressed immunoreactivity for fast skeletal muscle myosin (h, red), while only a few muscle fibres were immunoreactive for slow skeletal muscle myosin (i, red) in both non-trained and trained legs (h). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

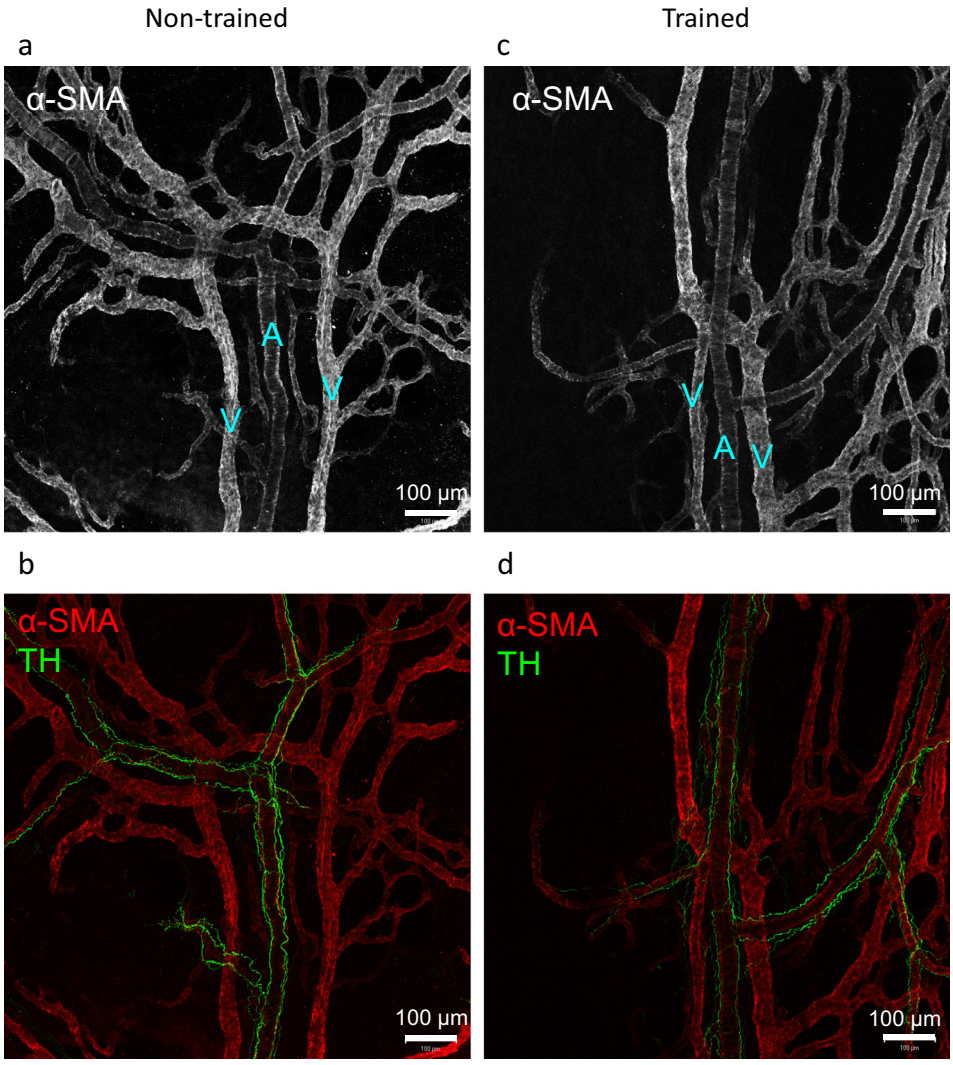

Fig. 2. Morphological characteristics of microvasculatures and perivascular sympathetic nerves in the tibial periosteum
Immunoreactivity for α-smooth muscle actin (α-SMA) revealed the microvasculature network of a tibia periosteum in non-trained leg (a). In the same specimen, sympathetic nerve fibres were revealed by their immunoreactivity for tyrosine hydroxylase (TH) (b). In trained leg of the same guinea-pig, α-SMA immunoreactive microvasculature network of tibia periosteum was visualised (c). In the same specimen, TH-positive perivascular sympathetic nerve fibres were demonstrated (d). An arteriole (A) and a venule (V) run parallel in both non-trained and trained legs. Perivascular sympathetic nerve fibres had a close apposition with arteriolar smooth muscle cells in both non-trained and trained legs.

not the vasoconstriction (n = 4, Fig. 4 h, i). Blockade of β-adrenoceptor with propranolol (1 μM) did not attenuate EFS-evoked vasoconstrictions (n = 4, Fig. 4k, l) or vasodilatation (n = 4, Fig. 4k, l).

3.7. Role of NO in nerve-evoked responses in periosteal arterioles

In periosteal arterioles of non-trained legs, L-nitro arginine (L-NA 100 μM), a nitric oxide synthase inhibitor, did not change the resting diameter of arterioles (22.1 ± 1.1 μm in control, 22.0 ± 2.0 μm in L-NA, P > 0.05, n = 5). However, EFS-evoked constrictions were enhanced in the arterioles that had been pretreated with L-NA (n = 5, Fig. 5a,b, c).

In periosteal arterioles of trained legs, L-NA (100 μM) did not change the resting diameter of arterioles (26.1 ± 3.0 μm in the diameter in control, 26.2 ± 3.2 μm in the diameter in L-NA, n = 5, P > 0.05). However, L-NA enhanced the EFS-evoked constriction (n = 5, Fig. 5g), and almost abolished the vasodilatation (n = 9, Fig. 5g).

Consistently, immunoreactivity for endothelial nitric oxide synthase (eNOS) was detected in tibial periosteum arterioles in both non-trained (Fig. 5d) and trained (Fig. 5h) legs. Immunoreactivity for eNOS was also detected in tibial periosteum venules of both non-trained (Fig. 5d) and trained (Fig. 5h) legs.

3.8. NAd-induced responses of periosteal arterioles and their modulation by nitric oxide

In periosteal arterioles of non-trained legs, bath-applied NAd (1 μM) caused a sustained constriction (n = 7, Fig. 6a). In contrast, NAd caused a phasic constriction of the arteriolar from trained legs (n = 10, Fig. 6d), with arteriolar diameter returning close to the basal level during NAd application. NAd (10 nM, 100 nM and 1 μM) induced arteriolar constrictions in a concentration-dependent manner in both non-trained (n = 7, Fig. 6c) and trained legs (n = 10, Fig. 6f).

Pretreatment with L-NA did not affect the NAd-induced arteriolar constrictions in non-trained legs (Fig. 6b,c). In contrast, L-NA enhanced the peak constrictions induced by 100 nM or 1 μM NAd in arterioles from trained legs and also converted the NAd-induced phasic constrictions into sustained constrictions (Fig. 6e,f).

4. Discussion

This study for the first time demonstrates that 10 weeks strength training of unilateral tibialis anterior muscle of guinea-pig using EMS protocol induced a sympathetic vasodilatation in periosteal arterioles of the trained leg but not the contralateral 'non-trained' leg.

Endurance and strength of the tibialis anterior muscle was remarkably increased in trained leg but not non-trained leg, suggesting that 10 weeks of EMS training protocol selectively changed the contractility of the targeted muscle. The increased fatigue resistance of

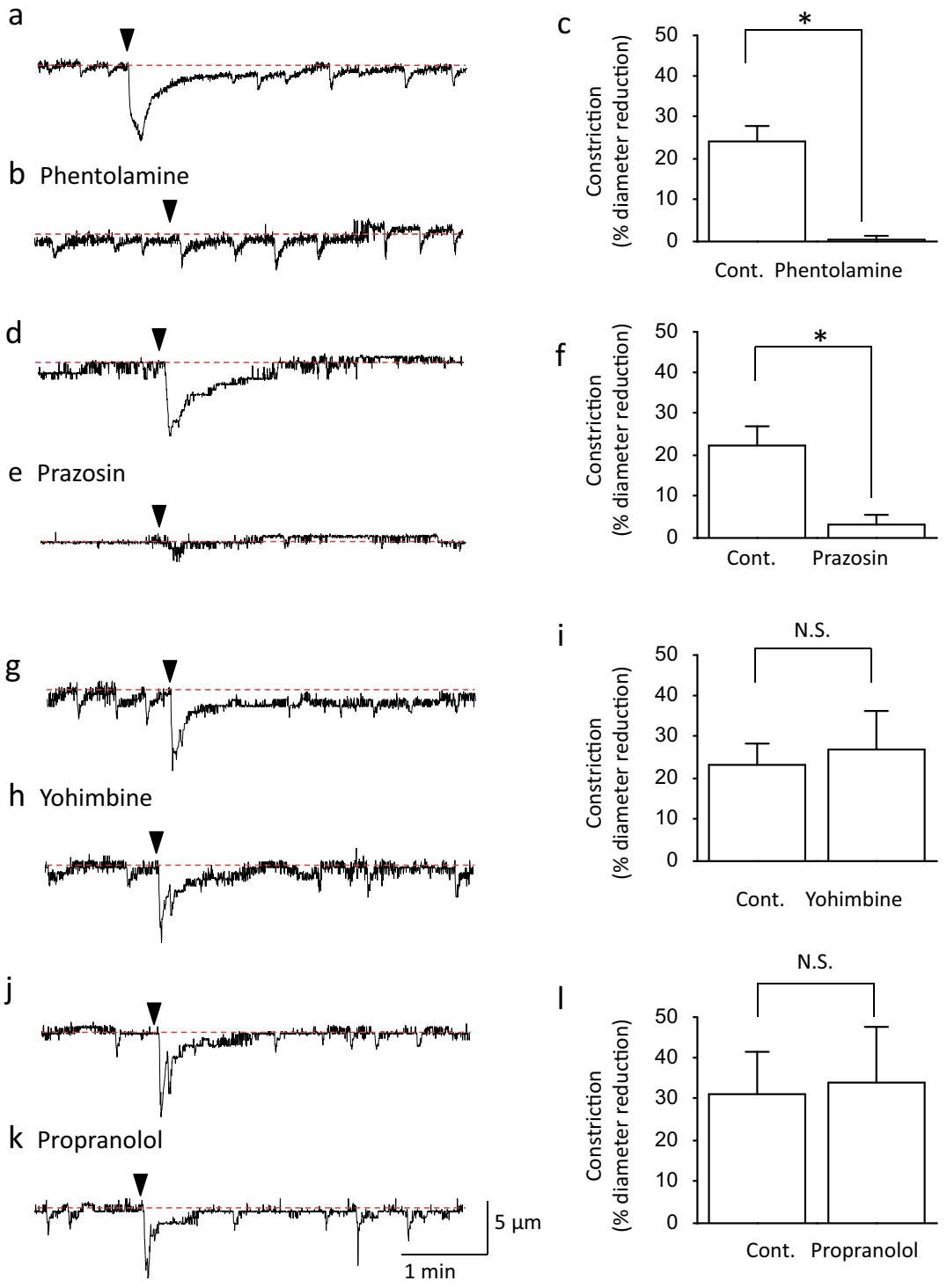

Fig. 3. Sympathetic nerve-mediated constriction of periosteal arterioles in non-trained leg
Electrical field stimulation (EFS, 100 µs duration, 10 Hz, 10 s) induced a phasic constriction in a periosteal arteriole (a). In the same arteriole, which had been treated with the non-selective α-adrenergic receptor antagonist phentolamine (1 µM), EFS failed to induce a constriction (b). These results were summarized in (c). Phentolamine ($n = 7$, $^*P < 0.05$) significantly attenuated EFS-evoked constriction. In another periosteal arteriole, EFS induced a phasic constriction (d). In the same arteriole, which had been treated with prazosin (1 µM), EFS-induced constriction was largely suppressed (e). These results were summarized in (f). Prazosin ($n = 6$, $^*P < 0.05$) significantly attenuated EFS-evoked constriction. Yohimbine (1 µM, n = 5, $^*P > 0.05$), did not suppress EFS-evoked constriction (g-i). Propranolol (1 µM, $n = 3$, $^*P > 0.05$), did not affect EFS-evoked constriction (j-l). The scale bars in (k) refer to all traces. Arrowheads indicate EFS.

tibial anterior muscle in trained leg is not detectable change in type I muscle fibres immunoreactive for slow skeletal muscle myosin that are responsible for the fatigue resistance in skeletal muscle (Kugelberg, 1973; Pette et al., 1975; Rochester et al., 1995). Thus, the increased muscle endurance may be predominantly due to changes in the properties of type II fast fibres. Endurance capacity results from numerous factors, including capability of oxygen supply to muscle, muscular energy resynthesis, and lactate removal (Pette et al., 1975). Species

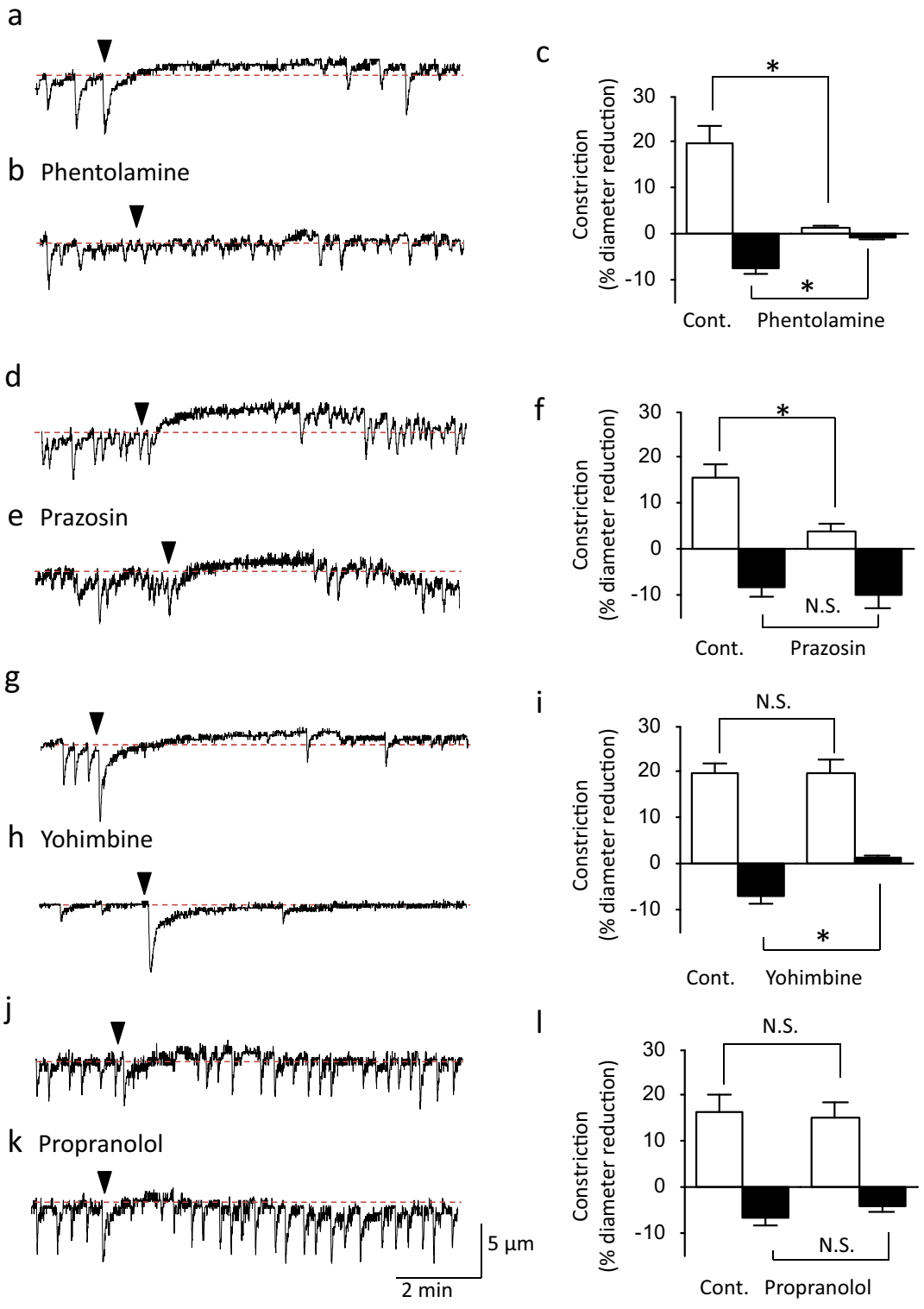

Fig. 4. Sympathetic nerve-mediated constriction and dilatation of periosteal arterioles in trained leg
Electrical field stimulation (EFS, 100 µs duration, 10 Hz, 10 s) induced a phasic constriction followed by a sustained dilatation in a periosteal arteriole (a). In the same arteriole, which had been treated with the non-selective α-adrenergic receptor antagonist phentolamine (1 µM), EFS failed to induce either a constriction or a sustained dilatation (b). These results were summarized in (c). Open bars indicate the EFS-induced constrictions, and closed bars indicate the EFS-induced dilatations. Phentolamine (n = 10) significantly attenuated both EFS-evoked constriction (open bar, $^*P < 0.05$) and dilatation (closed bar, $^*P < 0.05$). Prazosin (1 µM, n = 5), significantly attenuated EFS-evoked constriction ($^*P < 0.05$) but not dilatation (d-f). Yohimbine (1 µM, n = 6), significantly attenuated EFS-evoked dilatation ($^*P < 0.05$) but not constriction (g-i). Propranolol (1 µM, n = 4), did not affect either EFS-evoked constriction or dilatation. The scale bars in (k) refer to all traces. Arrowheads indicate EFS.

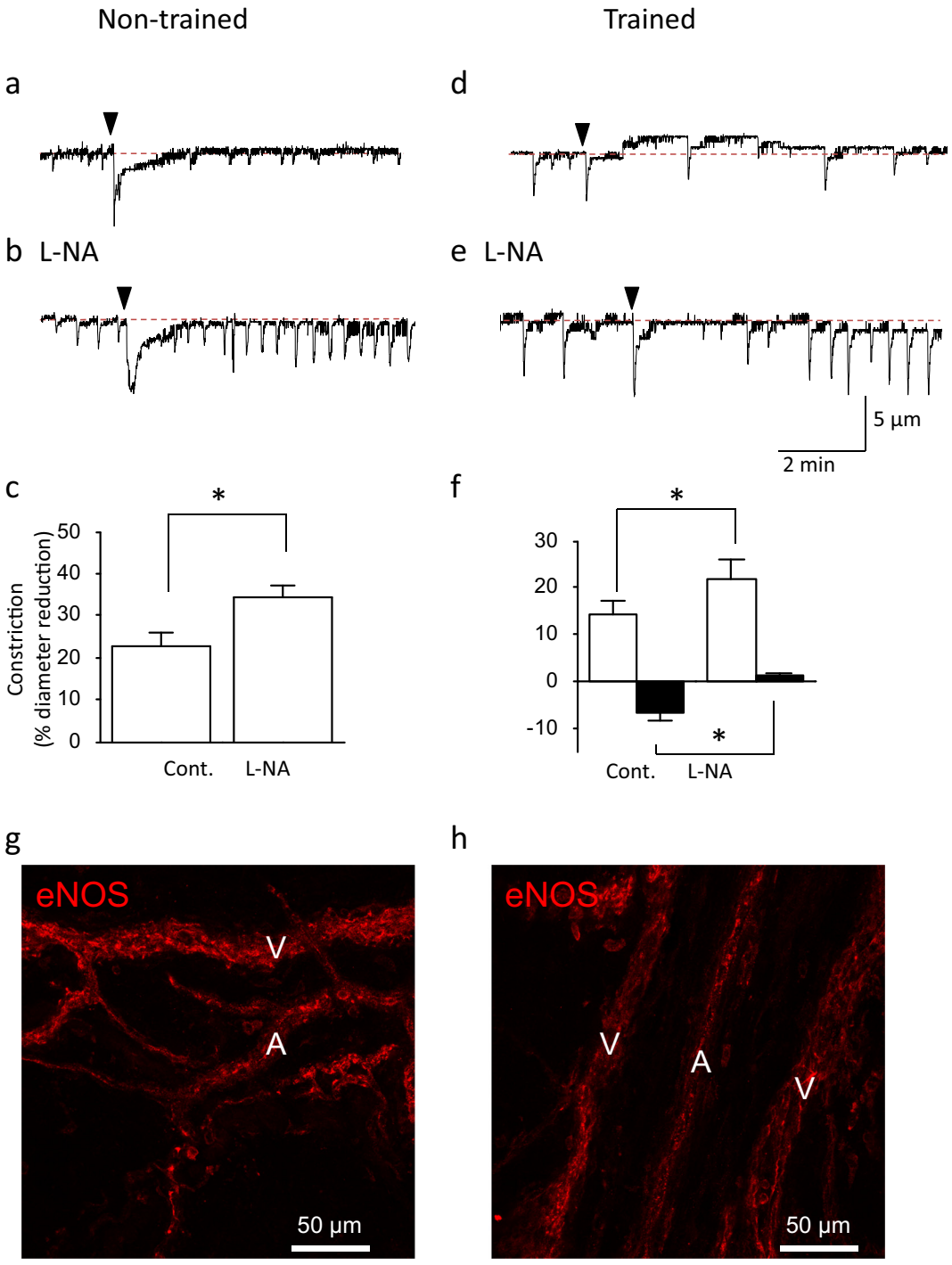

Fig. 5. Training-induced up-regulation of nitrergic inhibition on the contractility of periosteal arterioles
In a periosteal arteriole in non-trained leg, EFS (100 µs duration, 10 Hz, 10 s) induced a constriction (a). In the same arteriole, pre-treatment with L-nitroarginine (L-NA, 100 µM), a nitric oxide synthase inhibitor, enhanced the EFS-evoked constriction (b). These results were summarized in (c). L-NA (n = 5) significantly enlarged the EFS-evoked constrictions (*$P < 0.05$). In a periosteal arteriole in trained leg, EFS induced a phasic constriction followed by a sustained dilatation (d). In the same arteriole, which had been treated with L-NA (100 µM), EFS-induced constriction was enhanced, and EFS-induced dilatation was largely suppressed (e). These results were summarized in (f). L-NA significantly enlarged the EFS-evoked constrictions (open bar, $n = 11$, *$P < 0.05$) and suppressed the EFS-evoked dilatations (closed bar, *$P < 0.05$). The scale bars in (e) refer to all traces. Arrowheads indicate EFS.
Immunoreactivity for endothelial nitric oxide synthase (eNOS) was observed in an arteriole (A) as well as venules (V) of the periosteum in both non-trained (g) and trained (h) legs.

specific patterns of metabolic adaptation to increased contractile activity on type II fast fibres were reported (Simoneau and Pette, 1988). Skeletal muscles of smaller animals including guinea-pigs, in which increases in enzyme activity of aerobic-oxidative metabolism are evident, are better endowed for aerobic-oxidative metabolism compared with skeletal muscles of larger animals (Simoneau and Pette, 1988). Consistent with the increased endurance in trained but not non-trained contralateral muscle, sympathetic vasodilatation was observed only in the arterioles of trained side leg, suggesting that humoral substances circulating in the bloodstream was not involved in the training-induced

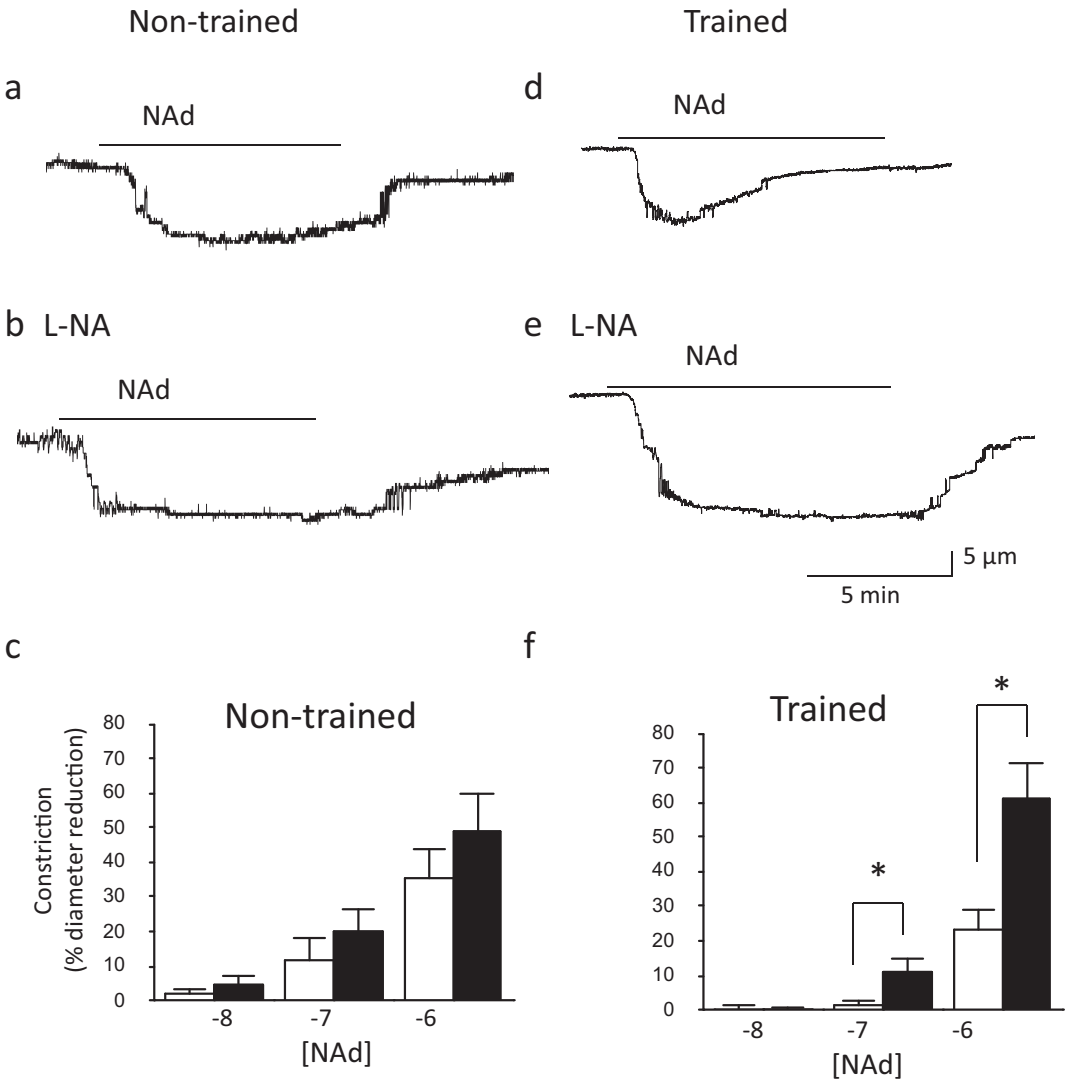

Fig. 6. Comparisons of noradrenaline-induced arterial constrictions between non-trained and trained legs
In a periosteal arteriole in non-trained leg, noradrenaline (NAd, 1 µM) induced a sustained constriction (a). In the same arteriole, NAd-induced sustained constriction was not changed by L-NA (b). In a periosteal arteriole in trained leg, NAd (1 µM) induced a phasic constriction. Thus, arteriolar diameter was gradually returned to the original level even during NAd application (d). In the same arteriole that had been pretreated with L-NA, NAd induced a sustained constriction with an increased amplitude (e). The peak amplitude of NAd (10 nM–1 µM)-induced constrictions in the absence (open bars) and presence (closed bars) of L-NA in non-trained leg (c) and trained leg (f) were summarized. Pre-treatment with L-NA increased the peak amplitude of NAd (100 nM or 1 µM)-induced constriction in trained legs ($^*P < 0.05$) but not non-trained legs. The scale bars in (e) refer to all traces.

changes.

4.1. Role of sympathetic innervation

Neuronal regulation of bone metabolism has previously been investigated based on anatomical analysis and cell culture studies (Imai and Matsusue, 2002). In the rat, most TH-containing nerve fibres in the bone are localized on the blood vessel wall, suggesting that the sympathetic innervation predominantly regulates vascular contractility. Consistently, blood volume in the bone is decreased upon sympathetic nerve stimulation in the dog (Davies et al., 1984). In the present study, varicose sympathetic fibres immunoactive for TH were found along the periosteal arterioles in both trained and non-trained legs of guinea-pigs. Since the EFS-induced constriction was abolished by α-adrenoceptors antagonist phentolamine or α_1-adrenoceptors antagonist prazosin, sympathetic constriction of the periosteal arterioles in guinea-pig appears to be exclusively mediated by NAd released from sympathetic nerves binding to α_1-adrenoceptors. Nevertheless, sympathetic co-neurotransmitters, e.g., ATP or neuropeptide Y (NPY), may have synergic interactions with NAd to facilitate sympathetic neurotransmission.

In the periosteum of trained legs, EFS-induced dilatation followed a transient constriction of the arterioles. Since the EFS-induced dilatation was abolished by α_2-adrenoceptors antagonist yohimbine but not propranolol, sympathetic dilatation of the periosteal arterioles in guinea-pigs appears to be induced by the binding of neuronally-released NAd to α_2-adrenoceptors and not β-adrenoceptors.

4.2. Role of endothelial NO

In the periosteal arterioles of the trained leg, L-NA enhanced the EFS-induced constrictions and also largely diminished the EFS-induced dilatations. In addition, phasic contractions induced by bath-applied NAd were converted into sustained contractions by pretreatment with L-NA in trained side arterioles, suggesting that NO appears to play critical roles in the sympathetic arteriolar dilatation as well as the

blunted sympathetic vasoconstrictions. In non-trained leg arterioles, bath-applied NAd produced sustained constrictions even in the absence of L-NA, suggesting NAd is no capable of stimulating NO release. Nevertheless, NAd-induced constrictions tended to be larger with L-NA, and thus there may be some basal NO production.

NAd-induced contractions of the rat abdominal aorta is blunted in an endothelial NO-dependent manner following moderate-intensity exercise training for 10 weeks (Burla et al., 2003; Delp et al., 1993), suggesting that endothelial NO-mediated suppression of sympathetic vasoconstriction could systemically be enhanced following exercise training. In the present study, the sympathetic dilatation of periosteal arterioles observed in trained leg arterioles appears to result from the elevated coupling of endothelial α_2-adrenoceptors to stimulate NO release.

In precontracted canine pulmonary and systemic blood vessels studied in vitro, NAd stimulates α_2-adrenoceptors located on the vascular endothelium to cause NO-mediated relaxation (Miller and Vanhoutte, 1985). During NAd stimulation, α_1-adrenoceptors-mediated vascular contraction is attenuated by NO released from the endothelium upon endothelial α_2-adrenoceptors stimulation (Vanhoutte and Miller, 1989). Furthermore, training-induced decreased sensitivity of the rat abdominal aorta to the vasoconstrictor effects of NAd appears to be due to an endothelium-dependent mechanism involving α_2-adrenergic receptors (Delp, 1995). In the present study, yohimbine greatly diminished nerve-evoked dilatation of the periosteal arterioles from trained leg, and thus it is likely that NAd binds to α_2-adrenoceptors on endothelium to cause NO release resulting in vasodilatation.

In the present study, pretreatment with L-NA enhanced the EFS-induced arteriolar constrictions not only trained but also non-trained legs. In trained side arterioles, endothelial NO release appears to be enhanced predominantly by NAd released from sympathetic nerves. Our previous study demonstrated that nerve fibres immunoreactive for substance P run along the periosteal arterioles in the guinea-pig (Fukuta et al., 2017). Consistent with the morphological observation, EFS-evoked arteriolar constrictions were attenuated by the stimulation of TRPV1-expressing primary afferent nerves using capsaicin or L-NA, while being enhanced by spantide, a substance P antagonist, suggesting that substance P released from primary afferents counteracts the sympathetic arteriolar constrictions by causing endothelial NO release (Fukuta et al., 2017). Thus, L-NA induced enhancement of the EFS-induced arteriolar constrictions is likely due to the inhibition of substance P-induced NO release in non-trained side arterioles.

5. Conclusion

Periosteal arterioles of the guinea-pig tibia develop sympathetic vasodilatation after a series of unilateral strength training of tibialis anterior muscle. The sympathetic vasodilatation in trained leg arterioles appears to be due to an enhanced α_2-adrenergic receptor-mediated, endothelial NO release resulting in the relaxation of arteriolar smooth muscle. Such training-induced changes in periosteal microvascular function would facilitate bone blood supply to meet the increased metabolic demand and may partially contribute to the exercise-induced increase in bone strength.

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