

# H-Reflex and Motor Responses to Acute Ischemia in Apparently Healthy Individuals

Donald W. Zakutansky,\* Koichi Kitano,† Janet P. Wallace,\* and David M. Koceja‡

**Abstract:** The authors examined the effect of acute ischemia on peripheral nerve function in healthy subjects. It was hypothesized that acute ischemia would interfere with the ability of sensory and motor nerves to propagate an impulse. Twelve young, apparently healthy adults participated in the study. Soleus H-reflex and motor recruitment curves were determined for subjects during a control condition, after 5 minutes of ischemia by femoral artery occlusion, and after a 5-minute recovery. During ischemia, the stimulus intensity required to evoke an H-reflex or M-wave was reduced by 18.3% and 18.4%, respectively.  $H_{\max}/M_{\max}$  ratios were significantly reduced with acute ischemia (mean  $\pm$  standard error)  $66.29\% \pm 5.4\%$  and  $58.81\% \pm 6.7\%$  for control and ischemia, respectively, owing to a decrease in  $H_{\max}$  during acute ischemia with no change in  $M_{\max}$ . After ischemia, the  $H_{\max}/M_{\max}$  returned to control values, as did the M-threshold. However, although the H-threshold slightly recovered, it failed to return to control threshold after 5 minutes of recovery. The results suggest that acute ischemia decreases motor and H-reflex thresholds in healthy individuals with a longer lasting effect for the H-reflex. In addition, a decrease in  $H_{\max}/M_{\max}$  ratio was observed, suggesting that acute ischemia has differential effects on sensory nerve propagation and synapse transmission.

**Key Words:** H-reflex, Excitability, Muscle, Nerve.

(*J Clin Neurophysiol* 2005;22: 210–215)

Ischemia is a syndrome that elicits nerve dysfunction in disease states. For instance, diabetic peripheral neuropathy is anatomically characterized by decreased endoneurial capillary density, thickened capillary basement membranes, and thickened endothelial cells leading to significantly reduced oxygen diffusion capacity (Malik et al., 1989). It has been suggested that diabetic peripheral neuropathy may be caused both from direct hyperglycemia-induced damage to the nerve

and from neuronal ischemia and hypoxemia caused indirectly by hyperglycemia-induced decreases in neurovascular flow (Tesfaye et al., 1994). Compelling evidence suggests the latter is at least partially responsible for peripheral nerve dysfunction (Malik et al., 1989; Forst et al., 1997). Acute morphologic changes in endoneurial microvessels have been seen in response to experimental ischemia in rats (Benstead et al., 1990). In addition, electrophysiologic studies supporting the hypoxemia hypothesis have been performed in rats living in hypoxic conditions (Low et al., 1985; Hendriksen et al., 1992). These studies reveal marked slowing of motor and sensory nerve conduction.

Electrophysiologic studies designed to describe the role of acute ischemia in nerve dysfunction have resulted in more sensitive markers of ischemia-induced nerve changes than simple nerve conduction velocities in humans (Weigl et al., 1989). Decreases in motor and sensory thresholds have been reported to occur rapidly, within 2 to 3 minutes, during ischemia produced by blood pressure cuff occlusion (Weigl et al., 1989). Such immediate decreases in both motor and sensory thresholds would indicate an acutely sensitive response; however, this response is limited to the axonal length, and does not uncover any information about alterations in synapse transmission with ischemia. In humans, H-reflex methodology is a useful tool to examine both the motor and sensory (Ia fibers) thresholds and changes in amplitude of the EMG signals produced by the percutaneous stimulation of both motor and sensory axons. Whereas the excitation of motor axons uncover the ability of the motoneuron axon to deliver a muscle twitch, the excitation of the Ia sensory fibers produce a muscle twitch via a monosynaptic synapse in the spinal cord to alpha motoneurons. The simplicity of this system (e.g., one synapse structure) allows one to localize the effect of ischemia to a single synapse in the human spinal cord. Thus, the use of H-reflex methodology may help to uncover how ischemia may produce changes in threshold excitability and information transfer from peripheral sensory fibers directly to the motor neurons. To date, protocols using the H-reflex to examine ischemia have not been reported. The purpose of this study was to examine the effects of acute lower limb ischemia on both the motor and H-reflex

\*Clinical Exercise Physiology Laboratory, †Motor Control Laboratory, ‡Program in Neural Science, Indiana University, Bloomington, Indiana, U.S.A.

Address correspondence and reprint requests to Dr. Donald W. Zakutansky, Indiana University, Clinical Exercise Physiology Laboratory – HPER 070, School of Health, Physical Education, & Recreation, Bloomington, IN 47405, U.S.A.

Copyright © 2005 by Lippincott Williams & Wilkins  
ISSN: 0736-0258/05/2203-0210

recruitment curves of the soleus muscle in young, apparently healthy individuals.

## METHODS

### Subjects

The experiment was conducted on 12 young, apparently healthy subjects (8 men and 4 women aged  $23.6 \pm 2.2$  years; age range, 19–28 years; height,  $168.5 \pm 6.4$  cm; weight,  $76.8 \pm 6.7$  kg). All subjects gave informed consent to procedures as approved by the Indiana University Committee for the Protection of Human Subjects and reported no history of diabetes or neuromuscular deficits on a preliminary screening questionnaire. H-reflex and M-wave recruitment curves were determined for each subject while in a prone position at rest (control condition), after 5 minutes of occlusive ischemia, and 5 minutes after occlusion. Each subject was tested for approximately 1 hour on 1 day.

### General Experimental Procedure

#### H-Reflex Testing

The soleus H-reflex was measured in the right leg using a bipolar surface recording electrode (Ag-AgCl) with an intraelectrode distance of 2 cm. This electrode was placed over the soleus muscle fibers at a point midway between the distal fibers of the gastrocnemius and the proximal border of the Achilles tendon. Soleus H-reflexes were elicited with a 0.80-cm-diameter stimulating electrode placed in the popliteal fossa of the right leg. A 4-cm-diameter carbon dispersal pad was placed on the anterior aspect of the right knee just above the patella. A metal reference plate was attached to the distal fibula just above the malleolus. Measurements of maximal soleus H-reflex amplitude ( $H_{\max}$ ) and motor response ( $M_{\max}$ ) were recorded during the H-reflex and motor response recruitment curve for each subject under three experimental conditions in the prone position: during the control condition, after 5 minutes of acute ischemia, and after 5 minutes of postischemic recovery. To develop a recruitment curve at each time period, the current delivered to the tibial nerve was incrementally increased from zero until a maximal M-wave was produced. Four trials were recorded at each stimulus intensity, with each subject receiving approximately 80 to 100 trials at each experimental condition. Threshold for the H-reflex and M-wave was defined as 2.5% of the maximal H-reflex or M-wave amplitude (Hilgevoord et al., 1994). During ischemia, changes in excitability were measured by the difference in stimulus intensity required to evoke wave amplitudes seen at threshold during the resting period. As a result of this procedure, a decrease in stimulus intensity represented an increase in excitability; conversely, an increase in stimulus intensity represented a decrease in excitability.

### Ischemia

Ischemia was induced to the right leg with a pneumatic blood pressure thigh cuff placed proximal to the carbon dispersal pad before any electrophysiologic testing. After the resting H-reflex and motor response recruitment curve was obtained, the cuff was inflated to 50 to 60 mm Hg above resting systolic pressures and maintained for 5 minutes. Ischemia was confirmed when tissue oxygenation, as measured by near infrared spectroscopy, fell to 0% within 3 to 4 minutes in all subjects. After the 5 minutes of ischemia, a second recruitment curve was obtained. The cuff pressure was immediately released on completion of the curve. Within the first minute of recovery, the tissue oxygenation returned to baseline. A final recruitment curve was obtained (with deflated cuff in place) 5 minutes after release of occlusion.

### Data Storage and Treatment

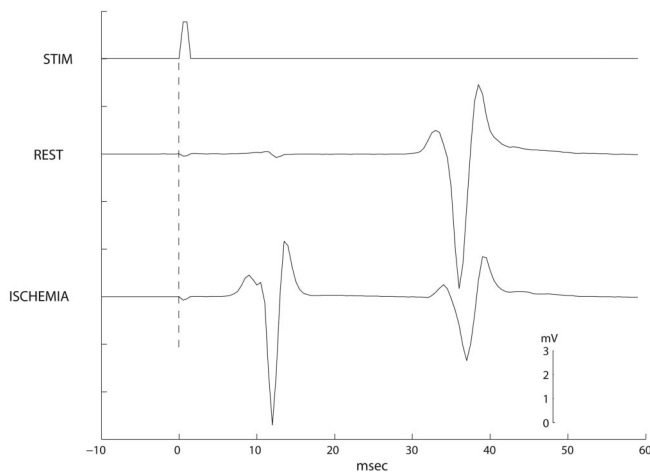
Electromyographic data were stored digitally using Acqknowledge Software, with a sample rate of 2 kHz (Biopac Systems, Goleta, CA, U.S.A.). The threshold measurement of the H-reflex and motor fibers was examined by the stimulus intensity necessary to elicit a threshold response in each fiber. The synapse transmission was measured with the  $H_{\max}/M_{\max}$  ratio for each experimental condition. Means for each measurement were compared with repeated-measures analysis of variance. Significant differences were examined with a Tukey post hoc analysis. The alpha level for all statistical tests was set at 0.05.

## RESULTS

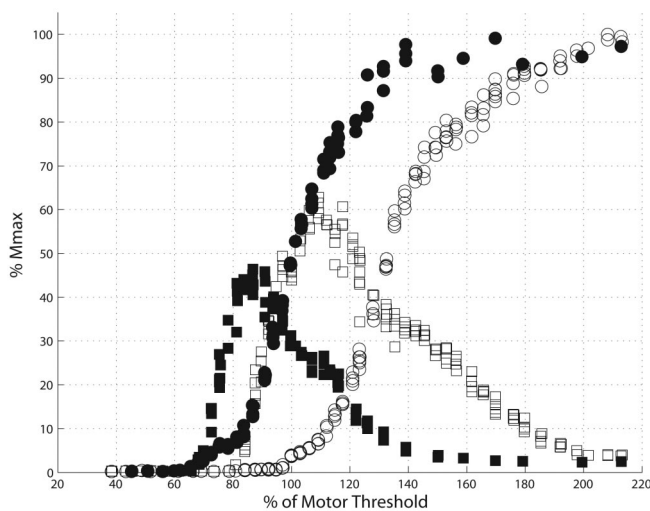
Individual traces for the M-wave and the H-reflex with a constant stimulus intensity for the control and ischemia conditions are shown in Fig. 1. With equal stimulus intensities, the M-wave was barely perceivable whereas there was a large-amplitude H-reflex during the control condition. During ischemia, the M-wave increased in amplitude, while the H-reflex decreased in amplitude. To explore this phenomenon in more detail, the individual recruitment curves were examined. Figure 2 depicts the recruitment curves for a typical subject; this figure demonstrates that the recruitment curves for both the H-reflex and the M-response were shifted to the left, consistent with increased excitability of the sensory and motor fibers.

### Motor and H-Reflex Threshold

To examine whether the excitability of the sensory and motor axons changed with ischemia, both motor and H-reflex thresholds in each condition were examined. Threshold for the H-reflex and M-wave was defined as 2.5% of the maximal H-reflex or M-wave amplitude. Threshold measurements were standardized to the stimulus intensity required to evoke motor threshold in the control condition (MTC). During control trials, the H-reflex threshold occurred at  $82.6\% \pm$



**FIGURE 1.** Responses of the M-wave and the H-reflex to the same stimulus intensity under control and ischemic conditions. Note that during control, a large-amplitude H-reflex is evoked, whereas during ischemia, an identical stimulus produced a large M-wave with a smaller H-reflex, suggesting a shift in excitability of the motor and sensory fibers.



**FIGURE 2.** The soleus H-reflex (triangles) and M-wave (circles) recruitment curves during control (filled) and acute ischemic conditions. Note the shift to the left along the abscissa (stimulus intensity) for both curves

3.1% (mean  $\pm$  standard error) of MTc. During ischemia, the H-reflex threshold occurred at  $67.5\% \pm 3.9\%$  of MTc, which significantly differed from the control value ( $F_{1,11} = 33.37$ ,  $P < 0.001$ ). The M-wave threshold during ischemia was also significantly reduced to  $81.6\% \pm 4.7\%$  of MTc ( $F_{1,11} = 14.64$ ,  $P = 0.003$ ). This represented an increased excitability of the H-reflex and motor axons as a result of ischemia. It is important to note that during recovery from ischemia (occlusion was removed), the excitability of the motor fibers was

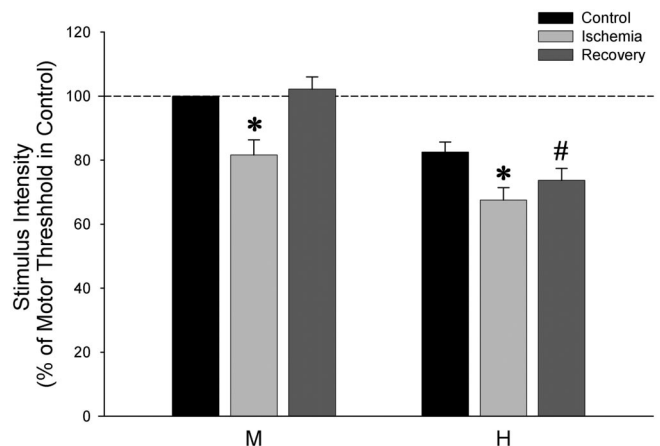
restored to control threshold values ( $102.2\% \pm 3.8\%$ ). The excitability of the H-reflex, however, slightly recovered but remained hyperexcitable compared with the control condition ( $67.5\% \pm 3.9\%$  to  $73.7\% \pm 3.7\%$ ). The H-reflex threshold after occlusion was removed was significantly greater than during the ischemic condition; however, it was also significantly decreased from the control condition. This change in threshold intensity is represented in Fig. 3.

### Synapse Transmission

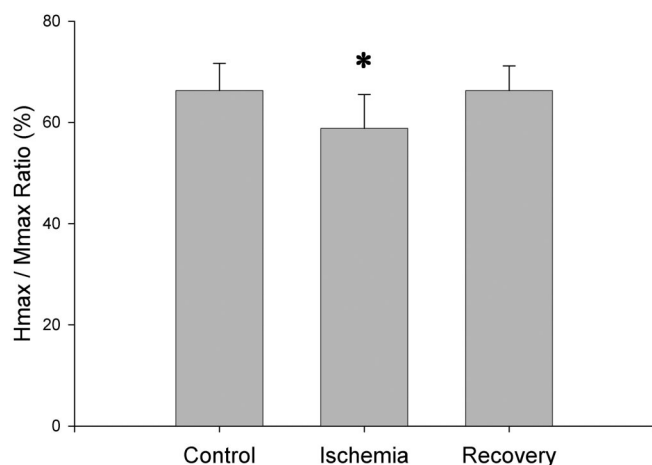
Sensory transmission across the Ia-alpha motoneuron synapse, as measured with the  $H_{\max}/M_{\max}$  ratio, was also altered with ischemia.  $H_{\max}/M_{\max}$  ratios were significantly reduced with acute ischemia ( $58.81 \pm 6.7\%$ ;  $F_{1,11} = 10.45$ ,  $P = 0.008$ ) when compared with control values ( $66.29 \pm 5.4\%$ ) or recovery values ( $66.27 \pm 4.9\%$ ) (Fig. 4). This reduced ratio was due to a significant decrease in  $H_{\max}$  amplitude ( $6.08 \pm 2.20$  mV versus  $5.18 \pm 2.18$  mV) during acute ischemia. There were no significant differences in the  $M_{\max}$  amplitude between control ( $9.85 \pm 4.28$  mV) and ischemic ( $9.68 \pm 3.98$  mV) conditions. Similar to the threshold changes, both the  $M_{\max}$  amplitude ( $10.19 \pm 1.24$  mV) and the  $H_{\max}$  amplitude ( $6.35 \pm 0.55$  mV) returned to control values after recovery.

### DISCUSSION

The results from this controlled ischemia experiment produced two important findings: (1) acute ischemia increased both motor and H-reflex excitability by decreasing motor and H-reflex thresholds in young, healthy individuals; and (2) this increased excitability was accompanied by a



**FIGURE 3.** Change in threshold for the motor response (M) and the H-reflex (H) between control and ischemic conditions. The asterisk denotes a significant change from control ( $P < 0.05$ ) during ischemia, whereas the number sign denotes a significant change from control to recovery. Note the decrease in M-threshold with ischemia and the decrease in H-threshold with ischemia and recovery.



**FIGURE 4.** The  $H_{\max}/M_{\max}$  ratio under control, ischemia, and recovery conditions. The  $H_{\max}/M_{\max}$  ratio was significantly reduced during ischemia ( $P < 0.05$ ) compared with the control condition.

decrease in  $H_{\max}$  amplitude. The increase in the excitability of the motor and sensory axons has previously been reported (Lin et al., 2002) and was an expected finding. However, the finding that this increased axonal excitability was accompanied by a decrease in synaptic transmission was unexpected. This provides evidence that acute ischemia has differential central nervous system effects: it increases axonal excitability in both motor and sensory nerves, but reduces synaptic transmission. Thus, we propose that two separate mechanisms must regulate each of these observations. It is important to note that this study examined acute ischemic changes in only young, healthy subjects, and inferences made to ischemia in disease states are, at best, made with caution because, unlike in this study, ischemia in disease is generally chronic and incomplete.

With respect to membrane threshold changes, Lundberg and Oscarsson (1953) stated that a period of anoxia induces membrane depolarization due to inhibition of the electrogenic  $\text{Na}^+$  pump and the subsequent  $\text{K}^+$  accumulation. On reintroduction of oxygen, membrane hyperpolarization ensues due to reactivation of the  $\text{Na}^+$  pump. The results of the current experiment are also consistent with the findings of Lin and colleagues (2002), who explored differences in responses to ischemia between human sensory and motor axons and determined sensory axons to have a greater decrease in threshold during ischemia. We found the stimulus intensity required to evoke the H-reflex threshold decreased 18.3% during ischemia while the M-wave threshold was evoked with an 18.4% reduction in stimulus intensity during acute ischemia. However, H-wave threshold failed to return to the control level of excitability, whereas the motor axons were fully recovered after 5 minutes of recovery. This cor-

roborates the notion that sensory fibers are more greatly affected by ischemia, and indicates that the sensory Ia fibers could be more dependent on pump activity than motor axons to maintain their resting membrane potential. This would explain the longer recovery time required to return to control threshold values of the H-reflex.

With respect to changes in the  $H_{\max}/M_{\max}$  ratio with acute ischemia, it was determined that  $H_{\max}$  was decreased, whereas  $M_{\max}$  was unaffected by ischemia. To explain this downregulation of the  $H_{\max}/M_{\max}$  ratio, we will examine the potential mechanisms in the reflex loop. As such, we propose the following potential mechanisms: (1) presynaptic inhibition to the Ia terminal, (2) changes in motoneuron membrane properties (e.g., as a result of indirect postsynaptic inhibition from group III-IV fibers), (3) intrinsic property changes in the Ia fiber, and (4) changes in the neuromuscular junction and muscle properties.

With respect to presynaptic inhibition of the Ia terminals, there is ample evidence to suggest that the Ia fiber does not have a fixed and invariant effect on the motor pool, but rather that transmission across this synapse can be altered by the environment (Chalmers and Knutzen 2002; Edamura et al., 1991; Koceja et al., 1993; Llewellyn et al., 1990) and by training (for review see Koceja et al., 2004). Presynaptic inhibition has been argued to allow for greater and lesser amounts of neurotransmitter to be released at the Ia-motoneuron synapse independent of the firing rates of the Ia fibers (Rudomin and Schmidt 1999). Moreover, modulation in these presynaptic interneurons has been observed before human voluntary movements, suggesting a central role in movement control (Hultborn et al., 1987a,b). Thus, it appears logical that ischemia may act to influence presynaptic interneurons and downregulate the effect of the Ia fiber onto the motor pool. This may be viewed as a mechanism whereby the central nervous system attempts to offset the increased excitability of the incoming sensory fiber activity with a concomitant increase in presynaptic inhibition to these fibers. This type of central nervous system adjustment has been observed in the reflex system in response to physical training (Hakkinen and Komi 1983a,b) and during the early stages of motor learning (Llewellyn et al., 1990). Because there exists an ample number of H-reflex protocols in the literature designed to measure presynaptic inhibition in humans (for review see Stein, 1995), perhaps the effect of ischemia on presynaptic interneurons will be examined in the future.

Another alternative explanation for the decreased  $H_{\max}/M_{\max}$  ratio is that perhaps ischemia produces a hyperpolarized motoneuron. For example, such an effect could be the result of group III-IV polysynaptic inhibition to the motoneurons, and the documented oligosynaptic influences on the H-reflex (Burke et al., 1984). The net result of motoneuron hyperpolarization would be a less excitable motor pool, and consequently a lower  $H_{\max}/M_{\max}$  ratio with ischemia. This

mechanism, however, seems unlikely in the present study. When comparing control and ischemic conditions, during ischemia the stimulus intensity necessary to evoke the same amplitude H-reflex was *decreased*. Assuming that the firing rates of the Ia fibers were similar between control and ischemic conditions (as a result of the 1-millisecond stimulus), a hyperpolarized motoneuron soma and dendrites would have resulted in greater current being necessary to elicit an H-reflex, not less current, as we observed. Therefore, the role of motoneuron membrane shifts with ischemia seems unlikely.

There has also been considerable interest in Ia-fiber intrinsic properties regulating the efficiency of this synapse in humans (Hultborn et al., 1998). Intrinsic properties refer to the firing rate of the Ia fiber and the collective effects of this firing rate on neurotransmitter release. Subjects in this study were tested lying prone, with little or no background electromyographic activity in the soleus muscle, and no movement of the limb. Thus, the role of spontaneous firing of Ia fibers on the present results seems negligible. Also, the H-reflex stimulus (1-millisecond pulse) produced a synchronous volley of Ia activity that was relatively independent of intrinsic effects.

It is also possible that neuromuscular junction and/or muscle membrane properties could account for changes in the  $H_{\max}/M_{\max}$  ratio observed in this study, and these should not be ignored. As such, potential explanations for the observed decrease in the  $H_{\max}/M_{\max}$  ratio during ischemia could likely be a deficiency in acetylcholine release at the neuromuscular junction (Tombol et al., 2002) or a decrease in muscle membrane potential (Perry et al., 1984). In rats subjected to 2 hours of ischemia, significant degradation of synaptic vesicles and the presynaptic membrane was evident (Tombol et al., 2002). Although this could occur in human models and may explain a decrease in  $H_{\max}$  with ischemia, this seems unlikely in the current study because there was no concurrent change in  $M_{\max}$ . Ischemia may also produce changes in the muscle membrane potential, accounting for changes in the excitability of the reflex loop. A decrease in muscle membrane potential with ischemia has been demonstrated in animal studies (Perry et al., 1984). Although this may help explain the excitability changes observed in the present study, these changes are unlikely to cause a reduction in the  $H_{\max}/M_{\max}$  ratio because a change in muscle membrane potential would not have selective effects on the H-reflex and motor response. In this study, the decrease in the  $H_{\max}/M_{\max}$  ratio was due solely to a change in  $H_{\max}$  with  $M_{\max}$  remaining unchanged. One possible source of  $H_{\max}$  amplitude decreases with ischemia we are unable to rule out is the increased effects of ischemia directly under the cuff. Changes in threshold current and latency are reported to be more pronounced at the site of ischemic compression (Bostock et al., 1991). Although changes in maximal amplitudes were not reported, we cannot dismiss the possibility.

In conclusion, it has been demonstrated that acute ischemia of the lower limb produced a peripheral excitability in both the H-reflex and motor axons, but that this increased axonal excitability was accompanied by a decrease in the efficiency of the Ia-fiber motoneuron synapse. The mechanisms responsible for these dichotomous results remain to be explored, but we propose two distinct explanations. Increased excitability of axons is due to an inhibition of the electrogenic  $\text{Na}^+$  pump and the subsequent  $\text{K}^+$  accumulation, and decreased synapse transmission is due to increases in presynaptic inhibition of Ia-fiber terminals.

## REFERENCES

- Benstead TJ, Sangalang VE, Dyck PJ. (1990) Acute endothelial swelling is induced in endoneurial microvessels by ischemia. *J Neurol Sci* 99:37–49.
- Bostock H, Baker M, Reid G. (1991) Changes in excitability of human motor axons underlying post-ischaemic fasciculations: evidence for two stable states. *J Physiol* 441:537–57.
- Burke D, Gandevia SC, McKeon B. (1984) Monosynaptic and oligosynaptic contributions to human ankle jerk and H-reflex. *J Neurophysiol* 52:435–48.
- Chalmers GR, Knutzen KM. (2002) Soleus H-reflex gain in healthy elderly and young adults when lying, standing, and balancing. *J Gerontol A Biol Sci Med Sci* 57:B321–9.
- Edamura M, Yang JF, Stein RB. (1991) Factors that determine the magnitude and time course of human H-reflexes in locomotion. *J Neurosci* 11:420–7.
- Forst T, Pfutzner A, Bauersachs R, et al. (1997) Comparison of the microvascular response to transcutaneous electrical nerve stimulation and postocclusive ischemia in the diabetic foot. *J Diabetes Complicat* 11:291–7.
- Hakkinen K, Komi PV. (1983a) Changes in neuromuscular performance in voluntary and reflex contraction during strength training in man. *Int J Sports Med* 4:282–8.
- Hakkinen K, Komi PV. (1983b) Alterations of mechanical characteristics of human skeletal muscle during strength training. *Eur J Appl Physiol Occup Physiol* 50:161–72.
- Hendriksen, PH, Oey PL, Wieneke GH, van Huffelen AC, Gispen WH. Hypoxic neuropathy versus diabetic neuropathy. An electrophysiological study in rats. *J Neurol Sci* 1992;110:99–106.
- Hilgevoord AA, Koelman JH, Bour LJ, Ongerboer de Visser BW. Normalization of soleus H-reflex recruitment curves in controls and a population of spastic patients. *Electroencephalogr Clin Neurophysiol* 1994;93:202–8.
- Hultborn H, Conway BA, Gossard JP, et al. (1998) How do we approach the locomotor network in the mammalian spinal cord? *Ann N Y Acad Sci* 860:70–82.
- Hultborn H, Meunier S, Morin C, Pierrot-Deseilligny E. (1987a) Assessing changes in presynaptic inhibition of Ia fibres: a study in man and the cat. *J Physiol* 389:729–56.
- Hultborn H, Meunier S, Pierrot-Deseilligny E, Shindo M. (1987b) Changes in presynaptic inhibition of Ia fibres at the onset of voluntary contraction in man. *J Physiol* 389:757–72.
- Koceja DM, Davison E, Robertson CT. (2004) Neuromuscular characteristics of endurance- and power-trained athletes. *Res Q Exerc Sport* 75:23–30.
- Koceja DM, Trimble MH, Earles DR. (1993) Inhibition of the soleus H-reflex in standing man. *Brain Res* 629:155–8.
- Lin, CS, Kuwabara S, Cappel-Smith C, Burke D. Responses of human sensory and motor axons to the release of ischaemia and to hyperpolarizing currents. *J Physiol* 2002;541(Pt 3):1025–39.
- Llewellyn M, Yang JF, Prochazka A. (1990) Human H-reflexes are smaller in difficult beam walking than in normal treadmill walking. *Exp Brain Res* 83:22–8.
- Low PA, Nukada H, Schmelzer JD, Tuck RR, Dyck PJ. (1985) Endoneurial oxygen tension and radial topography in nerve edema. *Brain Res* 341:147–54.
- Lundberg A, Oscarsson O. (1953) Anoxic depolarization of mammalian nerve fibres. *Acta Physiol Scand Suppl* 111:99–110.

- Malik RA, Newrick PG, Sharma AK, et al. (1989) Microangiopathy in human diabetic neuropathy: relationship between capillary abnormalities and the severity of neuropathy. *Diabetologia* 32:92–102.
- Perry MO, Shires III GT, Albert SA. (1984) Cellular changes with graded limb ischemia and reperfusion. *J Vasc Surg* 1:536–40.
- Rudomin P, Schmidt RF. (1999) Presynaptic inhibition in the vertebrate spinal cord revisited. *Exp Brain Res* 129:1–37.
- Stein RB. (1995) Presynaptic inhibition in humans. *Prog Neurobiol* 47:533–44.
- Tesfaye S, Malik R, Ward JD. (1994) Vascular factors in diabetic neuropathy. *Diabetologia* 37:847–54.
- Tombol T, Pataki G, Nemeth A, Hamar J. Ultrastructural changes of the neuromuscular junction in reperfusion injury. *Cells Tissues Organs* 2002; 170:139–50.
- Weigl P, Bostock H, Franz P, Martius P, Muller W, Grafe P. (1989) Threshold tracking provides a rapid indication of ischaemic resistance in motor axons of diabetic subjects. *Electroencephalogr Clin Neurophysiol* 73:369–71.