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INVITED REVIEW

ABSTRACT: Neuropathy is a common complication of end-stage kidney disease (ESKD), typically presenting as a distal symmetrical process with greater lower-limb than upper-limb involvement. The condition is of insidious onset, progressing over months, and has been estimated to be present in 60%–100% of patients on dialysis. Neuropathy generally only develops at glomerular filtration rates of less than 12 ml/min. The most frequent clinical features reflect large-fiber involvement, with paresthesias, reduction in deep tendon reflexes, impaired vibration sense, muscle wasting, and weakness. Nerve conduction studies demonstrate findings consistent with a generalized neuropathy of the axonal type. Patients may also develop autonomic features, with postural hypotension, impaired sweating, diarrhea, constipation, or impotence. The development of uremic neuropathy has been related previously to the retention of neurotoxic molecules in the middle molecular range, although this hypothesis lacked formal proof. Studies utilizing novel axonal excitability techniques have recently shed further light on the pathophysiology of this condition. Nerves of uremic patients have been shown to exist in a chronically depolarized state prior to dialysis, with subsequent improvement and normalization of resting membrane potential after dialysis. The degree of depolarization correlates with serum K⁺, suggesting that chronic hyperkalemic depolarization plays an important role in the development of nerve dysfunction in ESKD. These recent findings suggest that maintenance of serum K⁺ within normal limits between periods of dialysis, rather than simple avoidance of hyperkalemia, is likely to reduce the incidence and severity of uremic neuropathy.


UREMIC NEUROPATHY: CLINICAL FEATURES AND NEW PATHOPHYSIOLOGICAL INSIGHTS

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End-stage kidney disease (ESKD) occurs when nephrons are irretrievably impaired to the extent that the retention of metabolic waste products, salt, and water becomes potentially fatal. ESKD may occur due to either a primary renal disorder or as a complication of a multisystem disorder. The common causes of ESKD remain diabetes, glomerulonephritis, and hypertension. When renal function reaches critically low levels, renal replacement therapy either in the form of dialysis or transplantation is required in order to remove waste products and excess fluid.

Uremic neuropathy has for many decades been recognized as a common complication of ESKD, although the advent of dialysis and transplant programs has led to reductions in the rate of severe neuropathy, the prevalence of this condition remains high. This review covers the clinical and electrophysiological features of uremic neuropathy, with a focus on advances in understanding the pathophysiology of this condition.

HISTORICAL ASPECTS

The possibility of peripheral neuropathy in patients treated with hemodialysis was first raised shortly after the introduction of the first formal hemodialysis program. The first clinical documentation of neuropathy was provided in 1961 in two young male patients with hereditary interstitial nephritis and deafness. The development of neuropathy in these cases, how-
ever, was attributed to the underlying hereditary disor-
der, rather than viewed as a complication of ESKD.

Following this report, Asbury et al.\textsuperscript{6} provided
extensive clinical and pathological findings in four
men who developed neuropathy as a consequence of
ESKD of varying etiologies. All four patients had
clinical features of renal disease for many years be-
fore the development of neuropathy, which mani-
ifested as a symmetrical length-dependent sensorimo-
tor neuropathy. Nerve biopsies established axonal
degeneration, maximal distally, with sparing of prox-
nal nerve segments and nerve roots. Moreover,
there was no evidence to suggest nerve compression,
inflammation, or the superimposition of a systemic
disease process, such as diabetes or amyloid, leading
to the conclusion that the development of neurop-
athy was a consequence of the underlying renal dis-
order.

Early clinical neurophysiological investigations in
ESKD patients demonstrated reductions in motor
nerve conduction velocity in symptomatic and
asymptomatic patients.\textsuperscript{158,185} Jebsen et al.,\textsuperscript{82} studying
the natural history of uremic neuropathy, compared
clinical and nerve conduction findings in patients
treated conservatively to those receiving dialysis ther-
apy. Whereas the development of neuropathy in the
conservatively treated group was related to deterio-
rating renal function, those patients treated with
long-term dialysis manifested improvement in both
clinical and neurophysiological parameters. Follow-
ing these early reports and in light of the increasing
use of dialysis and renal transplantation therapies,
greater attention has been focused on uremic neu-
ropathy, with numerous studies reporting high rates
of neuropathy in ESKD patients, generally relating
the development of neuropathy to the severity of
renal failure.\textsuperscript{5,20,82,107,144,146,149,158,185,187} Of particular
note, studies by Nielsen\textsuperscript{144,145} and Bolton et
al.\textsuperscript{20,21} in the 1970s demonstrated nerve conduction
slowing in clinically unaffected nerve segments, with
correlation between the extent of renal impairment
and degree of conduction slowing, as well as im-
provement in neurophysiological parameters follow-
ing renal transplantation. These studies provided
clinical evidence to suggest that a uremic toxin was
responsible for the development of neuropathy in
ESKD patients, a hypothesis that was to become a
major focus of future neurophysiological research in
this condition.

**INCIDENCE AND CLINICAL FEATURES**

Peripheral neuropathy in ESKD generally presents
as a distal symmetrical polyneuropathy with greater
lower-limb than upper-limb involvement. The condi-
tion is of insidious onset, progressing over months,
and has been noted to have a male predominance. It
generally only develops at glomerular filtration rates
of less than 12 ml/min.\textsuperscript{39} The most frequent clinical
features are those of large-fiber involvement, with
paresthesias, reduction in deep tendon reflexes,
impaired vibration sense, weakness, and muscle wast-
ing (Fig. 1). In the 1970s, Nielsen\textsuperscript{144,145} demonstrated
the presence of neuropathic symptoms in
over 50% of patients with ESKD. Other studies have
demonstrated prevalence rates varying from 60% to
100%, depending on the diagnostic criteria ap-
plied.\textsuperscript{1,25,122,191}

Laaksonen et al.\textsuperscript{122} staged the clinical severity of
uremic neuropathy in 21 ESKD patients, using a
modified version of the neuropathy symptom score
(NSS) developed by Dyck et al.,\textsuperscript{57,58} and combined
this assessment with results of nerve conduction studies. The NSS quantified symptoms that were grouped into three categories to reflect alteration in motor, sensory, and autonomic systems. Within each group, further subsets were used to group symptoms according to the region affected and the presence of positive or negative symptoms. Using the NSS and the staging procedure previously used in studies of diabetic patients,57,58 81% of ESKD patients received a diagnosis of neuropathy. Stage 1 neuropathy (asymptomatic neuropathy) was diagnosed in 19%, stage 2 neuropathy (symptoms nondisabling) was present in 48%, and stage 3 neuropathy (disabling symptoms) was noted in 14%. In a more recent study,113 93% of ESKD patients had neuropathic symptoms on NSS testing, with 72% diagnosed with stage 2 neuropathy and 21% with stage 3 neuropathy, despite all patients meeting currently accepted guidelines of dialysis adequacy.143

CLINICAL AND NEUROPHYSIOLOGICAL FINDINGS IN GENERALIZED UREMIC NEUROPATHY

Early studies of uremic neuropathy utilizing nerve biopsy techniques revealed prominent axonal degeneration, most severe in the distal parts of nerve trunks. Although initial studies suggested that demyelination was a significant feature of uremic neuropathy,5,54 subsequent reviews demonstrated that demyelination was secondary to axonal loss and that proximal segments of the nerves were relatively spared.3,55,67,187 These findings supported the concept that uremic neuropathy was a dying-back neuropathy, with metabolic failure of the neuron causing distal axonal degeneration.25

Numerous neurophysiological series have been undertaken in patients with uremic neuropathy and have demonstrated findings consistent with a generalized neuropathy of the axonal type.1,4,13,20,41,46,49,68,80,107,113,120,130,148,155,156,179,183,185,191 Early studies focused on motor nerve conduction parameters and demonstrated slowing of conduction velocity in patients prior to the development of clinical neuropathy.25,158 Subsequent studies demonstrated abnormalities of nerve conduction148 with generalized slowing in both sensory and motor nerves, accompanied by reduction in sensory response amplitudes. Motor response amplitudes tend to remain relatively preserved, although abnormalities in lower-limb motor nerves were noted in some patients, accompanied by neurogenic changes in distal lower-limb muscles on electromyography.148,149

In a recent study, amplitude of the sural sensory nerve action potential was found to be the most sensitive indicator of uremic neuropathy, being reduced in 50% of ESKD patients.113 Other groups have confirmed similar findings, demonstrating reductions in sensory and motor response amplitudes in addition to abnormalities of late responses.1,4,49,122,130,157,157,191 Reduction in peroneal motor conduction velocity46,157,148 and prolongation of tibial F-wave minimum latencies122 have been established as sensitive indicators of neuropathy in ESKD patients. Prolongation of soleus H reflexes has also been demonstrated in patients without clinical evidence of neuropathy, suggesting that this parameter may be more sensitive in detecting early neuropathy.68,191

Studies of quantitative sensory testing in ESKD patients have demonstrated increased vibratory perception thresholds, most marked in the lower limbs,147 whereas somatosensory-evoked potentials in ESKD patients demonstrate abnormalities of conduction along both the distal and proximal segments of peripheral somesthetic pathways, but less commonly along intracranial sensory pathways.25,155,166 A study of single-fiber electromyography demonstrated normal fiber densities in motor units of ESKD patients.186 This finding suggested that reinnervation, characterized by increased fiber density, had failed to occur. However, this was accompanied by increased jitter, possibly reflecting peripheral demyelination in the setting of axonal degeneration. A further single-fiber EMG study established that jitter abnormalities improved following a year of dialysis.106

Early studies of nerve excitability, utilizing a limited range of excitability parameters, demonstrated an elevated threshold for excitation even when nerve conduction values were normal, in addition to demonstrating prolongation of absolute and relative refractory periods.25,125,182,208 As a consequence, it was concluded that the safety factor for neural transmission at the nodes of Ranvier would be lowered. Unexpectedly, uremic nerves retained vibratory perception and their sensory response amplitudes for a longer period than control nerves when rendered ischemic.43 Uremic nerves also behaved differently when temperature was lowered, with a less rapid rise in response amplitude compared to controls.24,25

In addition to the slowly progressive sensorimotor axonal neuropathy, a more rapidly progressive motor neuropathy has been described. A small number of ESKD patients with diabetes have also been shown to develop a subacute neuropathy progressing over a few months, with severe muscle weakness. In this group of patients, nerve conduction studies may demonstrate features of either a demyelinating
or axonal neuropathy.26,27,165 Although the presence of diabetes complicates assessment of nerve conduction data, the absence of preexisting neuropathic symptoms and the clinical improvement noted following dialysis or renal transplantation suggest a metabolic basis for the neuropathy, related to the underlying ESKD. Analysis of cerebrospinal fluid (CSF) is rarely helpful, as CSF protein concentration is frequently elevated in ESKD patients and may simulate the albuminocytologic dissociation that is characteristic of Guillain–Barré syndrome.25

Small-fiber neuropathy may develop as a clinical entity in ESKD patients. Lindblom and Tegner124 demonstrated abnormalities of thermal sensation in 30% of ESKD patients and concluded that small-fiber neuropathy may exist as a distinct entity in these patients. These results, however, differed from those of other groups who demonstrated minimal impairment of thermal sensation in ESKD.56,188 In a study of 20 ESKD patients, abnormalities in standard nerve conduction studies were demonstrated in 16 patients,4 whereas abnormal thermal thresholds were found in only 6 patients and, when present, did not correlate with clinical evidence of polyneuropathy.4 Such findings are consistent with those of pathological studies that demonstrated greater vulnerability of larger-diameter fibers in ESKD patients.55

MONONEUROPATHIES IN ESKD

Mononeuropathies are a frequent clinical complication in ESKD patients and most typically occur in the median, ulnar, and femoral nerves.39

Carpal tunnel syndrome (CTS) is the most common mononeuropathy in ESKD, with prevalence rates varying from 6% to 31%.15,17,52,66,67,76,81,170 β₂-Microglobulin amyloidosis is a major factor underlying the development of CTS in ESKD patients.63 A complication noted in patients on long-term hemodialysis. Amyloid deposits have been identified in synovial specimens from dialysis patients with CTS63 and an increase in the rate of CTS has been demonstrated with increasing hemodialysis duration.63 Strategies geared at reducing the levels of β₂-microglobulin, such as the use of high-flux biocompatible membranes and β₂-microglobulin adsorption columns, have resulted in reduced rates of CTS development and ultimately improvement in symptoms.63,118,192

Other factors that may contribute to the increased incidence of CTS in ESKD patients include uremic tumoral calcinosis17,203 and the placement of arteriovenous fistulas (Fig. 2), inducing a “steal” of blood from the distal limb.66,70,105,119,131,141,201 The placement of Brescia–Cimino arteriovenous fistulas between the radial artery and cephalic vein has been associated with the development of both the clinical features of CTS and subclinical neurophysiological abnormalities in median or ulnar nerve territories.70,105 A recent study demonstrated CTS in 30.5% of limbs with fistulas compared to 12.2% on the contralateral side.66 Although the site of the fistula had no effect on the development of CTS, a significant correlation was noted between the age of the fistula and the presence of CTS.

With regard to treatment of CTS in ESKD, the outcome of median nerve decompression appears inferior in this group compared to patients with idiopathic CTS.76,102,173 In addition, recurrence rates are higher. In ESKD patients with recurrent CTS, an endoscopic approach may prove to be effective in relieving persistent symptoms.211

Ulnar neuropathy is also a common occurrence in ESKD, affecting up to 51% of patients undergoing hemodialysis.142 Causes include external compres-
sion at the elbow during prolonged dialysis therapy and other risk factors as outlined for CTS, particularly arteriovenous fistulas and uremic tu- moral calcinosis.

In addition to their possible contributions to the development of CTS and ulnar neuropathy, upper-limb arteriovenous fistulas may be associated with an acute-onset neuropathy, first described by Bolton et al. and later termed ischemic monomelic neuropathy. In this condition, acute limb ischemia develops due to shunting of arterial blood away from the distal parts of the limb. The severity of ischemia typically affects nerves, without causing changes in other tissues such as muscle, that possess a higher threshold for ischemic injury. Diabetic patients are particularly vulnerable, especially those with preexisting peripheral vascular disease or neuropathy. The symptoms are those of multiple upper-limb mononeuropathies, with distal sensory loss and weakness in the muscles of the forearm and hand. Electromyography demonstrates neurogenic abnormalities in distal limb muscles with sparing of proximal musculature. There may be predilection for median nerve involvement and the presence of conduction block in this nerve has been described as a sign of reversible injury. Early ligation or revision of the fistula frequently leads to significant clinical and electrophysiological improvement.

Femoral neuropathy in ESKD patients is a well-recognized complication of renal transplantation, with an incidence of – 2%. Prolonged use of self-retaining retractors in the region of the femoral nerve during renal transplantation may cause nerve compression and neuropathic injury. Rapid recovery is expected in cases of neurapraxia but prolonged ischemia may lead to axonal loss. The prognosis is generally favorable, with most patients achieving complete recovery, although residual deficits may be present when significant axonal loss has occurred.

AUTONOMIC NEUROPATHY IN ESKD

Autonomic neuropathy may develop in ESKD patients, manifesting as postural hypotension, impaired sweating, diarrhea, constipation, or impotence. In a study of 36 ESKD patients, gastrointestinal autonomic symptoms were evident in 42% and impotence in 45%. Although postural hypotension was an uncommon clinical finding, 36% complained of episodes of postural dizziness, which was most prominent in elderly ESKD patients. Some studies have suggested that autonomic neuropathy occurs as a manifestation of generalized polyneuropathy, but others have shown no correlation between autonomic dysfunction and peripheral nervous system abnormalities. The mechanisms underlying the development of uremic autonomic neuropathy remain unknown, although an association with hyperparathyroidism has been suggested.

Studies utilizing objective measures of autonomic function, including R–R interval variation as a measure of parasympathetic function and sustained handgrip and sympathetic skin response as measures of sympathetic function, have established abnormalities in up to 62% of ESKD patients on dialysis treatment. However, these abnormalities frequently occur in the absence of clinical symptoms of autonomic dysfunction. Parasympathetic dysfunction has been shown to occur with greater frequency than sympathetic dysfunction, which is generally more common in diabetic ESKD patients.

The contribution of autonomic dysfunction to the development of intradialytic hypotension remains a matter of ongoing debate, with some studies suggesting a possible association and others suggesting no significant relationship. A recent review of the literature on the use of the oral alpha-1-adrenoceptor agonist midodrine in the treatment of intradialytic hypotension suggested a beneficial effect, although the authors drew attention to the fact that most studies were not randomized and had small sample sizes.

EFFECTS OF DIALYSIS AND TRANSPLANTATION ON UREMIC NEUROPATHY

Early reports investigating the effects of hemodialysis on uremic neuropathy suggested that some patients with mild neuropathy recovered completely with adequate dialysis. In fact, failure to improve was considered to be an indicator of insufficient dialysis. These reports, however, did emphasize that the extent of improvement was likely to be related to the severity of neuropathy and that patients with severe neuropathy were unlikely to experience any significant recovery.

More recent studies, however, have demonstrated that improvement in neuropathy with dialysis is an uncommon event. Although these studies suggest that dialysis retards the progression of neuropathy in most patients, in some cases a gradual deterioration of neuropathy may occur. A comparison of hemodialysis and peritoneal dialysis with regard to neuropathy pro-
progress has demonstrated no significant difference between the two dialysis forms.184

Renal transplantation remains the only known cure for uremic neuropathy,20,21 with clinical improvement in sensory and, to a lesser extent, motor function occurring within a few days of transplantation.80 Serial nerve conduction studies following transplantation demonstrated a correlation between the improvement in nerve conduction and biochemical parameters, suggesting that metabolic phenomena may underlie the rapid improvement.156 Even with severe neuropathy, improvement in symptoms and signs may occur within 1 month of transplantation, although in some patients the recovery is prolonged or remains incomplete.21,152,183

Dialysis and transplantation are less beneficial for patients with autonomic neuropathy compared to large-fiber neuropathy. An early study suggested that autonomic function may be improved with dialysis,74 but a more recent report failed to show any significant benefit.198 Although renal transplantation may lead to improvement or normalization of autonomic function,127,164 the time course of such improvement is often slow and may be incomplete, with significant changes often occurring after 4–8 years.177,199

Recent evidence suggests that treatment with erythropoietin (EPO) may prove beneficial in ESKD patients with neuropathy71,174 as well as for patients with neuropathy due to other etiologies.91,189 Treatment with EPO improved motor nerve conduction velocity in ESKD patients, but had no effect on sensory indices. In vitro studies have shown that EPO receptors are present on Schwann cells and in dorsal root ganglion neurons.79,90 Upregulation of EPO receptors occurs after axonal injury, mediated by release of nitric oxide, and administration of exogenous EPO is associated with reduction in limb weakness and neuropathic pain behavior.90

DIALYZABLE TOXINS AND THE MIDDLE MOLECULE HYPOTHESIS

Hegstrom et al.72 postulated that uremic neuropathy occurred due to accumulation of a dialyzable substance on the basis of their observational studies that demonstrated improvement in neuropathy in two subjects with long-standing ESKD following commencement of dialysis therapy. Later studies demonstrated that patients treated with peritoneal dialysis had lower rates of uremic neuropathy despite the fact that these patients frequently had higher blood urea and creatinine concentrations.10 The lower neuropathy rate in the peritoneal dialysis group was thought to indicate that the substance responsible for neuropathy was better dialyzed by the peritoneum than by the cellophane membranes used in hemodialysis. On this basis, the most likely group of substances was thought to be the “middle molecules,” substances with a molecular weight of 300–12,000 Da.193 given that such substances were known to be poorly cleared by hemodialysis membranes.

Marked elevations in the concentrations of middle molecules have been demonstrated in ESKD patients, a finding not observed in healthy controls.60,61 Examples of such molecules include parathyroid hormone (PTH) and β-2-microglobulin (β-2M), the levels of which are elevated in patients with ESKD.195 Further studies demonstrated that the use of thinner dialysis membranes and longer dialysis times, strategies that would have greater benefits for the clearance of middle molecules compared to small molecules, led to significant reductions in the rates of severe neuropathy.9,64 A study using a hemodialysis membrane highly permeable to middle molecules also demonstrated a dramatic reduction in the development of neuropathy.129

These early studies, however, were hampered by a number of difficulties, not least of which was the inability to measure middle molecule levels.10 Another major shortcoming of the hypothesis has been the lack of conclusive evidence that any single molecule in the middle molecular range is actually neurotoxic.104,198 In a study of nerve conduction velocity following renal transplantation, correlation was noted between the postoperative concentration of myoinositol, a middle molecule, and median nerve sensory conduction velocity.156 Although myoinositol levels are elevated in ESKD patients, there is little convincing evidence for a neurotoxic effect.195,207

The only middle molecule for which some evidence of neurotoxicity exists is PTH, with some studies suggesting a link between PTH and the neurological complications of ESKD.132,176 PTH has been shown to prolong motor nerve conduction velocities in animal studies,55 although human studies of the effect of PTH on peripheral nerves have yielded conflicting results, with variable changes in motor nerve conduction velocity in patients with ESKD.8,53,169

Despite the shortcomings of the middle molecule hypothesis, the hypothesis that a dialyzable toxin may be involved in the pathophysiology of this condition remains prevalent. More recently, it has been suggested that the following criteria should be met in order for a substance to be truly regarded as a uremic neurotoxin: (1) it must be an identifiable chemical; (2) it should be elevated in the blood of uremic patients; (3) there should be a direct positive relationship between blood level and neurological
dysfunction; (4) it should cause neurological dysfunction in animals at appropriate blood levels; and (5) its removal from the blood should abolish the dysfunction. The middle molecule hypothesis fails to satisfy a number of these criteria, most importantly criterion 3, as there is very little evidence to suggest that such molecules are actually neurotoxic.

Despite the evidence that a dialyzable toxin may underlie the development of uremic neuropathy, the mechanism of this neurotoxicity remained unclear. The possibility that the neurotoxic effect may be due to alteration in membrane excitability was first proposed by Nielsen who, drawing on evidence from in vitro studies of muscle and red blood cells in ESKD patients, proposed that one or more of these toxins may cause neuropathy by inhibiting activity of the axonal Na⁺/K⁺ pump. This energy-dependent pump is electrogenic, with three Na⁺ ions being pumped out for every two K⁺ ions pumped into the axon, leading to a net deficit of positive charge on the inner aspect of the axonal membrane. Paralysis of the Na⁺/K⁺ pump abolishes the direct contribution of the hyperpolarizing pump current to the membrane potential and leads to an accumulation of extracellular K⁺ that causes further depolarization. The Na⁺/K⁺ pump is therefore of critical importance in maintaining normal ionic gradients, which are essential for axonal survival. Disruption of these gradients may cause reverse operation of the Na⁺/Ca²⁺ exchanger, leading to increased levels of intracellular Ca²⁺ and axonal loss.

Although it is not possible to measure membrane potential directly in human axons in vivo, indirect information regarding membrane potential and axonal ion function may be gained from nerve excitability studies. Axonal excitability can be investigated using threshold tracking, where “threshold” indicates the stimulus current required to produce a target potential that can be adjusted online by computer (i.e., tracked) to assess excitability. The recent development of automated protocols has facilitated the use of excitability techniques in the clinical setting. Rather than relying on a single parameter, excitability techniques provide information regarding alterations in membrane potential and axonal ion channel function based on coherent changes in a number of different indices. Nerve excitability measures have been used to study peripheral nerves in patients with neuropathy and have provided information about disease pathophysiology. Prior to discussing the changes in axonal excitability that develop in patients with ESKD, a general understanding of nerve physiology is critical for the further discussion related to the pathophysiological mechanisms involved in the development of neuropathy.

**MOLECULAR STRUCTURE OF THE AXON AND SALTATORY CONDUCTION**

Transmission of impulses in myelinated axons occurs by means of saltatory conduction (Fig. 3), with action potentials advancing between successive nodes of Ranvier:

*Like a kangaroo travelling at speed, the action potential advances at near-uniform velocity, but it

![Figure 3](image-url)
is powered by discrete kicks of inward membrane current at nodes of Ranvier.\textsuperscript{75}5

The chief role of the axon is that of impulse conduction, which depends on the electrical cable structure and voltage-dependent ion channels of the axonal membrane. Much of the knowledge about axonal membrane structure and ion channel function comes from studies in nonmammalian axons (squids, vertebrates). Only over the last few decades have techniques such as patch-clamping allowed investigation of mammalian axons, and only over more recent years have human axons been studied in vitro.

In myelinated axons from peripheral nerve, voltage-sensitive \(\text{Na}^+\) channels are clustered at high densities (up to \(1,000/\mu\text{m}^2\)) in the nodal axon, compared to the internodal region (\(25/\mu\text{m}^2\)).\textsuperscript{202} The high density of \(\text{Na}^+\) channels at the node reflects the need of saltatory conduction for a large inward current at the node (Fig. 3). When the nodal membrane is depolarized, an inward current is established, carried by \(\text{Na}^+\) ions. The \(\text{Na}^+\) conductance is voltage sensitive and regenerative: it increases with depolarization, and this in turn leads to greater depolarization as well as depolarization of the next node.\textsuperscript{75} This explosive process would end with the whole axon depolarized, were it not that the \(\text{Na}^+\) channels immediately started closing again, due to \(\text{Na}^+\) channel inactivation.

\(\text{Na}^+\) channels are membrane-spanning protein molecules, containing a pore unit (\(\alpha\)-subunit) through which \(\text{Na}^+\) ions can diffuse almost freely in the open state.\textsuperscript{171} The fast activation process (from resting closed to open state) and the slower inactivation process (from open to inactivated state) consist of conformational changes by the channel protein, both driven by the changes in voltage gradient across it. A variety of toxins and drugs bind to the \(\alpha\)-subunits of \(\text{Na}^+\) channels. Most toxins bind to sites that are involved in activation and inactivation, except for tetrodotoxin and its derivatives that occlude the outer pore of the \(\text{Na}^+\) channel.\textsuperscript{100,154} Some of these binding sites are also the target of mutation in hereditary \(\text{Na}^+\) channelopathies.\textsuperscript{101}

**ASSESSMENT OF NERVE EXCITABILITY IN A CLINICAL SETTING**

Assessment of nerve excitability using automated protocols (Fig. 4) includes assessment of both nodal and internodal axonal properties. The activity of nodal persistent \(\text{Na}^+\) conductances may be assessed by measurement of the strength–duration time constant and rheobase. The strength–duration time constant (\(\tau_{SD}\)) refers to the rate at which the threshold current for a target potential declines as the stimulus duration is increased.\textsuperscript{28,138,139,204} Calculation of \(\tau_{SD}\) in human subjects may be performed using the ratio between the stimulus–response curves for two different stimulus durations using Weiss’ formula,\textsuperscript{204} shown below for threshold currents for stimuli of 0.2 ms (\(I_{0.2}\)) and 1.0 ms (\(I_{1.0}\)) duration:

\[
\tau_{SD} = 0.2(I_{0.2} - I_{1.0})/[I_{1.0} - (0.2 \times I_{0.2})]
\]

Both strength–duration time constant and rheobase (the threshold for a stimulus of infinitely long duration) are properties of the nodal membrane, dependent on passive membrane properties and a local response mediated by persistent \(\text{Na}^+\) channels.\textsuperscript{86} Alterations in membrane potential will affect both parameters, with depolarization leading to prolongation of strength–duration time constant and reduced rheobase; and hyperpolarization causing a shorter strength–duration time constant and increased rheobase. When measured in isolation, strength–duration time constant, however, is of limited utility as a marker of membrane potential, as it is also affected by other factors including demyelination and discrete changes in nodal \(\text{Na}^+\) conductances.\textsuperscript{28,100}

Threshold electrotonus is the only clinical technique available to assess alterations in both nodal and internodal conductances. This method measures the threshold changes produced by long-duration depolarizing and hyperpolarizing currents.\textsuperscript{30,37} These conditioning polarizing currents are set to defined percentages of the unconditioned threshold current, but importantly remain subthreshold in that they do not trigger an action potential.\textsuperscript{37} The response of the threshold current to the subthreshold conditioning currents is tested at varying conditioning–test intervals before, during, and after the conditioning currents. By convention, threshold changes are plotted as threshold reductions (see Figs. 5, 6), such that depolarizing responses are plotted in an upward direction and hyperpolarizing responses in a downward direction. In addition to providing information regarding internodal conductances, threshold electrotonus is also sensitive to changes in axonal membrane potential,\textsuperscript{11,92} with membrane depolarization causing a “fanning-in” appearance of threshold electrotonus,\textsuperscript{86} whereas hyperpolarization leads to a “fanning-out” (Fig. 5).

Information regarding axonal ion channel function may also be gained through assessment of recovery cycle parameters. Following conduction of a single impulse, myelinated axons go through a stereotypical series of excitability changes known as the
recovery cycle (Figs. 5, 6). For a period of 0.5–1.0 ms after an impulse, axons are completely inexcitable and cannot generate another impulse regardless of the strength of the depolarizing stimulus. This period is known as the absolute refractory period. The axon then enters a period of relative refractoriness that can be measured either as the increase in current required to produce a potential of a specified size, known as refractoriness, or as the duration of the relative refractory period until threshold has returned to baseline, usually 3–4 ms. This period of refractoriness is followed by a period of superexcitability (or supernormality), during which there is a reduction in threshold occurring over a 10–15-ms interval. Finally, there is a late phase of raised threshold known as late subexcitability, ending around 100 ms. These changes in threshold are associated with changes in latency, which is increased during the refractory period, decreased during superexcitability, and increased during late subexcitability.16,181

The relative refractory period results from inactivation of nodal transient Na$^{+}$ channels. It is prolonged by membrane depolarization and reduced by hyperpolarization. Refractoriness may therefore be used as a measure of membrane potential, although it is essential to take into account the effect of temperature, given that cooling leads to an increase in refractoriness.94 Furthermore, measures of refractoriness may be unreliable in situations of impaired distal transmission, as may occur with axonal demyelination, neuromuscular junction abnormalities, muscle disease, or any other factor that reduces the security of impulse transmission.
Alteration in refractoriness may also occur secondary to changes in nodal \( \text{Na}^+ \) conductances. Reductions in refractoriness have been demonstrated in diabetic and toxic neuropathies, consistent with a reduction in the nodal \( \text{Na}^+ \) conductances. In a recent study, an increase in refractoriness was demonstrated in patients treated with the chemotherapeutic agent oxaliplatin, in the absence of significant changes in other excitability parameters, suggesting that...
the neurotoxicity of this agent may be mediated by blockage of nodal transient Na\textsuperscript{+} channels\textsuperscript{110}. Superexcitability is due to a depolarizing afterpotential that results from the capacitative charging of the internode by the action potential\textsuperscript{12} and subsequently discharges through high resistance pathways under or through the myelin sheath.\textsuperscript{40,99} Recent studies have also suggested that activation of nodal Na\textsuperscript{+} conductances may be a further contributing factor.\textsuperscript{133} As for refractoriness, superexcitability also varies with membrane potential, with depolarization leading to a reduction in superexcitability and hyperpolarization causing an increase. Late subexcitability is determined by activation of nodal slow K\textsuperscript{+} channels and the difference between membrane potential (E\textsubscript{m}) and the K\textsuperscript{+} equilibrium potential (E\textsubscript{K}) and increases with depolarization if extracellular K\textsuperscript{+} is unchanged. It may therefore be used to differentiate between pure depolarization and depolarization secondary to nerve ischemia in which there is no significant change in late subexcitability due to compensatory changes in extracellular K\textsuperscript{+} ions.\textsuperscript{92}

**NERVE EXCITABILITY STUDIES IN ESKD**

Nerve excitability studies in ESKD patients (Fig. 4) have recently demonstrated significant alterations in membrane potential prior to hemodialysis, with recovery in the postdialysis period.\textsuperscript{97,113,115–117} Prior to dialysis, measures of nerve excitability were significantly abnormal in ESKD patients compared to control data.\textsuperscript{93,95,108} Stimulus–response curves were shifted to the right, indicating that axons were of high threshold. This was accompanied by a fan-

**FIGURE 6.** Comparison of threshold electrotonus (A,C) and recovery cycle (B,D) in ESKD patients (continuous lines with circles) pre- and postdialysis, with 95% confidence intervals for normal controls (broken lines). Predialysis traces demonstrate “fanning-in” of threshold electrotonus, increased refractoriness, and reduced superexcitability and late subexcitability. Abnormalities have largely resolved 1 h postdialysis (reproduced with permission from Oxford University Press; Krishnan et al.,\textsuperscript{113} fig. 2).
Potassium satisfies criteria that have been suggested for a substance to be accepted as a uremic neurotoxin. It is an identifiable chemical that is elevated in the serum of ESKD patients and causes neurological dysfunction in both humans and animals. It is also a critical determinant of axonal resting membrane potential. Moreover, a direct relationship exists between serum levels of K⁺ and neurophysiological parameters, and its removal leads to considerable improvement in these indices. The resting potentials of both nerve and muscle membranes are largely determined by K⁺, with relative changes in K⁺ capable of depolarizing membranes.

It may be argued that the abnormalities of serum K⁺ noted in excitability studies are the consequence of a transient homeostatic disturbance, rapidly corrected by dialysis, and therefore unlikely to play a major role in the development of chronic irreversible neuropathy. Against such an argument, prolonged exposure to hyperkalemia in ESKD patients seems likely, given that postdialysis rebound of K⁺ is a well-recognized phenomenon with hyperkalemia typically recurring within 6 h of a dialysis session due to reequilibration between intracellular and extracellular fluid compartments. Such prolonged hyperkalemia may cause disruption of normal ionic gradients and activate damaging Ca⁺⁺-mediated processes, leading to axonal loss. Furthermore, given the importance of K⁺ in mediating these abnormalities, current measures of dialysis adequacy, which are based solely on blood urea concentrations, may be inappropriate for determining the adequacy of a dialysis regimen to prevent neurotoxicity. A better indication of adequate dialysis might be the maintenance of serum K⁺ within normal limits between periods of dialysis, which may require more attention to dietary restriction of K⁺ intake in some patients.

**AXONAL NA⁺/K⁺ PUMP FUNCTION IN ESKD PATIENTS**

Inhibition of the Na⁺/K⁺ pump by uremic neurotoxins, previously proposed as the mechanism underlying the development of uremic neuropathy, may induce membrane depolarization. Of further relevance, previous studies have demonstrated that alterations in membrane potential and intra- and extracellular K⁺ concentration have a direct effect on Na⁺/K⁺ pump function.

Nerve excitability measurements provide a means of detecting changes in membrane potential caused by activation of the Na⁺/K⁺ pump. Vagg et
al. showed that the activity-dependent hyperpolarization (ADH) of motor axons induced by voluntary activity causes threshold changes that can be used to assess Na\(^+/\)K\(^+\) pump function. Assessment of activity-dependent excitability changes prior to hemodialysis in 10 ESKD patients demonstrated quantitatively similar changes in ESKD patients and controls, arguing against any significant reduction in the axonal Na\(^+/\)K\(^+\) pump in ESKD (Fig. 8).115

A further study also assessed Na\(^+/\)K\(^+\) pump function in six ESKD patients prior to dialysis by monitoring the excitability changes that occurred before, during, and after 13 min of nerve ischemia.116 With the onset of ischemia, a short-lived threshold reduction was followed by a rapid increase in threshold (Fig. 9). Whereas normal controls manifested a postischemic threshold increase due to increased activity of the Na\(^+/\)K\(^+\) pump and consequent membrane hyperpolarization,112 a paradoxical reduction in threshold was noted in ESKD patients. This pattern suggested that the axonal Na\(^+/\)K\(^+\) pump had a stabilizing effect on the axon and was attempting to return membrane potential toward baseline from the highly depolarized levels of the ischemic period. Importantly, however, the rapid return of threshold to baseline levels in the postischemic period confirmed that the Na\(^+/\)K\(^+\) pump was functioning well in ESKD.

CONCLUSION

Neuropathy is a common complication of ESKD, occurring in the majority of patients undergoing dialysis. At present, renal transplantation remains the only known cure for uremic neuropathy.20,21 Recent nerve excitability studies have suggested that hyperkalemia may underlie the development of neuropathy and have argued against any dysfunction of the axonal Na\(^+/\)K\(^+\) pump in the development of this condition. Excess K\(^+\) fits the profile of the neurotoxin responsible for uremic neuropathy better than middle molecules, parathyroid hormone, or any other organic substance that has been previously linked to the development of uremic neuropathy. Recent findings from nerve excitability studies in ESKD patients suggest that maintenance of serum

![FIGURE 8. Comparison of excitability changes following maximal voluntary contraction in ESKD patients predialysis (filled diamond) and normal controls (open diamond). The filled arrow indicates the time of contraction. (A) Changes in normalized threshold for a 1-ms stimulus duration. (B) Strength–duration time constant, \(r_{SD}\). (C) Superexcitability. (D) Submaximal CMAP amplitude. Threshold and CMAP amplitude changes are normalized to precontraction values and are expressed as mean data \(\pm\) SEM. Although baseline superexcitability was significantly less in ESKD patients, there were no significant differences between patients and controls in the magnitude of excitability changes induced by activity,99 arguing against Na\(^+/\)K\(^+\) pump dysfunction in this condition (reprinted with permission from International Federation of Clinical Neurophysiology; Krishnan et al.,115 fig. 9).](#)

![FIGURE 9. Excitability parameters recorded from tibialis anterior for ESKD patients and controls before, during, and after the ischemic period (indicated by filled horizontal bar). Mean data \(\pm\) SEM. During ischemia refractoriness increased and superexcitability decreased in both groups, consistent with axonal depolarization. The reversal of these changes in the postischemic period is consistent with axonal hyperpolarization. There is a smaller threshold reduction during ischemia in ESKD patients and a prominent ischemic threshold increase beyond baseline values, a feature not observed in control subjects (reproduced with permission from Oxford University Press; Krishnan et al.,116 fig. 3).](#)
K+ within normal limits between periods of dialysis, rather than simple avoidance of hyperkalemia, is likely to reduce the incidence and severity of uremic neuropathy.

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INVITED REVIEW

ABSTRACT: The spectrum of motor neuron diseases ranges from disorders that clinically are limited to lower motor neurons to those that exclusively affect upper motor neurons. Primary lateral sclerosis (PLS) is the designation for the syndrome of progressive upper motor neuron dysfunction when no other etiology is identified. Distinction between PLS and the more common amyotrophic lateral sclerosis (ALS) relies primarily on recognition of their symptoms and signs, as well as on ancillary, although non-specific, laboratory data. In this review, we survey the history of PLS from the initial descriptions to the present. We discuss the role of laboratory, electrodiagnostic, and imaging studies in excluding other diagnoses; the findings from major case series of PLS patients; and proposed diagnostic criteria. Consistent differences are evident in patients classified as PLS compared to those with ALS, indicating that, despite its limitations, this clinical designation retains important utility.


PRIMARY LATERAL SCLEROSIS

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Since their first description over a century ago, motor neuron disorders (MND) have remained among the most intractable of neurologic ailments. Classically, they have been considered to span a clinical spectrum from exclusively lower motor neuron diseases, such as progressive muscular atrophy, to solely upper motor neuron disorders, such as hereditary spastic paraplegia and primary lateral sclerosis (PLS). The most common motor neuron disease, amyotrophic lateral sclerosis (ALS), falls in between, with both upper and lower motor neuron dysfunction.

Our advancing understanding of these disorders reveals the limitations of this classification and of distinctions based on age. ALS, initially thought to represent a selective degeneration of motor neurons, is now recognized to affect multiple cell types in the nervous system and to affect cognition as well as movement.63 Hereditary spastic paraplegia, most often a disease of youth, has subsequently been found to present in patients after age 50.45,49 Conversely, mutations in the alsin gene cause a juvenile form of PLS, which had previously been defined as manifesting in maturity.51

PLS is the focus of this review. That designation has long been used for the syndrome of progressive upper motor neuron dysfunction when no other cause can be identified. Over the years, refinement of diagnostic capabilities has enabled a variety of etiologies, from cobalamin deficiency to multiple sclerosis, to be excluded reliably, but a number of patients still remain with an unknown cause for their disorder.

We discuss here the characteristic manifestations and natural history of PLS. Recent studies have combined modern imaging approaches, electrophysiologic techniques, and laboratory studies to refine this challenging diagnosis. We review the major case series to help distinguish PLS from its more familiar and rapidly fatal counterpart, ALS. Ultimately, it is that crucial difference in prognosis that underlies the continued utility of this diagnosis.

HISTORICAL BACKGROUND

Jean-Martin Charcot first reported PLS in 1874, calling it "primary sclerosis of the lateral columns."54
Wilhelm Heinrich Erb’s contemporaneous description of the disease presentation remains instructive: “[PLS] usually begins slowly and insidiously... with some sense of weight, dragging and slight feebleness in one or the other leg, without pain... The condition progresses just as slowly as it commenced; the legs become stiffer and heavier, the gait progressively more labored, dragging, and distinctly spastic; occasional muscular cramps and contraction of the legs may occur, but nothing else. Objective examination reveals... the characteristic symptom triad: a certain weakness and awkwardness of movement of the legs, very slight, so that often for a long time nothing in the way of paresis, much less paralysis, is present; no ataxia— but far more distinct and prominent are the spasm and rigidity of the muscles...; and thirdly, the well-marked exaggeration of tendon reflexes... with involuntary clonus of the foot... while the patient is sitting.”

Charcot also noted the paucity of pathologic evidence and inability to know conclusively that lower motor neuron signs would not appear over time. These concerns, which made it difficult to categorize PLS as a distinct disease entity, still apply in the modern era. As he stated in a public lecture, “pathological investigations have not yet furnished any proof, and hence the solution to the problem remains in suspense. Meanwhile, the clinical description deserves to exist alone.”

Over the next half-century, a small number of reports followed. In 1905, Spiller described eight cases with autopsy data; of these, however, six demonstrated degeneration of neurons in the anterior horn and therefore should be classified as ALS. In 1943, Swank and Putnam published a large series of cases of motor neuron disease from the Neurological Institute of New York, including 19 potential cases of PLS. Two years later, Stark and Moersch described 43 cases seen over 22 years at the Mayo Clinic and suggested diagnostic criteria for PLS, which were utilized until the development of modern imaging and neurophysiologic testing.

The caution voiced by Charcot as to whether PLS is a distinct entity was reiterated by Kinnier Wilson in his authoritative textbook of neurology: “Divergent views are still held in regard to so-called primary lateral sclerosis, the ‘spastic spinal paralysis’ of Erb. Some consider it belongs to a separate class from Charcot’s disease [ALS], taking presence or absence of muscular atrophy for the criterion; but since ‘pure’ atonic atrophy often co-exists with slight pyramidal lesions only disclosed after death, spasticity without wasting might well represent the opposite extreme of the same condition.”

Renewed interest in PLS was stimulated by Fisher’s 1977 report of six cases of chronic progressive quadriplegia. He described one autopsy, in which selective degeneration was present in the corticospinal tract, without involvement of anterior horn cells or muscle. In 1981, Beal and Richardson presented a single case, with postmortem examination similarly demonstrating corticospinal degeneration and sparing of the anterior horns. Another report prior to the era of modern imaging came the following year, with the description by Russo of four living patients.

Younger et al. offered a description of three groups of living patients and results of three autopsies. The patients included six with exclusively upper motor neuron findings, seven who additionally demonstrated electromyographic (EMG) abnormalities (including one who went on to develop ALS), and two with underlying human immunodeficiency virus (HIV) infection. The investigators asserted the case for PLS as a distinct clinical entity. Their report framed key issues that have guided subsequent work.

In 1992, Pringle et al. described eight cases with one autopsy. They proposed what remain the current diagnostic criteria, broadened to include patients with limited electrophysiologic evidence of denervation. A number of relatively large series followed. In 2001, Le Forestier et al. reported 20 patients from the ALS Center at the Salpêtrière Hospital; they contended that most patients with a diagnosis of PLS show mild signs of lower motor neuron denervation on follow-up. A cohort of 25 patients was described by Zhai et al. at the National Institutes of Health. They subdivided the patients into an “ascending group” and a “multifocal group,” each with characteristic patterns of disease progression: the former displayed stereotypical advancement of symptoms from the lower extremities to the upper extremities and bulbar region; the latter showed an asymmetric or variable pattern of progression. More recently, Kuipers-Upmeijer et al. described 10 patients from Groningen, noting a variety of abnormalities including evidence of chronic denervation on EMG, prolonged central motor conduction times, abnormal somatosensory evoked potentials, and atrophy of the precentral gyrus. Singer et al. reviewed 25 cases from two University of Texas neuro-muscular clinics, with patients showing differing prognoses dependent on whether they demonstrated changes of active denervation on EMG. Gordon et al. reported on 16 patients, proposing refinements to the diagnostic criteria and more...
specific clinical subtypes. Insights from these studies are discussed further in what follows.

**EPIDEMIOLOGY AND DEMOGRAPHICS**

**How Often Is PLS Diagnosed?** Larger retrospective series have made it clear that PLS is an uncommon diagnosis among patients with motor neuron disease (MND). Swank and Putnam identified 19 possible cases of PLS in a population of 197 MND patients over 10 years, a frequency of nearly 10%.66 Stark and Moersch identified 43 cases from referrals to the Mayo Clinic over a 22-year period, deriving an incidence of 3.7 per 10,000 cases of organic neurologic disease.62 These early figures, however, were not based on specific inclusion or exclusion criteria.

More recent series estimate that 2%–5% of patients seen in adult neuromuscular clinics will be diagnosed with PLS. Pringle et al. identified 8 cases among 500 MND patients (1.6%) over a 10-year period.53 Le Forestier et al. reported 20 of 450 MND patients (4.4%) over 5 years and, in the University of Texas series, 25 PLS cases were identified from 837 MND patients over 5 years (3%).58

**Age at Onset.** As a disease of middle age or later onset, PLS usually can be distinguished from hereditary forms of spastic paralysis. Symptoms in PLS, as in ALS, typically begin in the fifth to sixth decade (Table 1). Recent series have reported mean age of onset ranging from 45.4 to 53.7 years.29,41,53,58,75 The range of disease onset is broad, however, and there is considerable overlap with age of onset of hereditary forms of spastic paraparesis (HSP), including a late-onset form.27,32 A juvenile-onset form of PLS has also been described.51

**Gender Distribution.** Some series have reported a marked male predominance, whereas in others both genders are similarly affected (Table 1). There may be a slight male predominance of PLS cases, although the numbers are drawn from small populations; overall, the distribution appears in the range expected for ALS, where there is a slight male predominance."72

**CLINICAL FEATURES**

The most common manifestations of PLS are leg weakness and spasticity, and spastic bulbar weakness (Table 1). As with ALS, sensory symptoms and signs should prompt continued investigation for an alternative diagnosis. Although upper-extremity symptoms frequently develop over time, they are uncommon as an initial manifestation. Rarely, patients experience progressive hemiparesis before spread to the opposite side becomes clinically apparent.27,32

It is unclear, however, whether exclusively limb or bulbar cases truly exist, or whether the disease would progress to complete spinobulbar spasticity given sufficient time. All eight patients in the Pringle series ultimately developed symmetric spinobulbar spasticity,53 and 29% of the limb-onset group in the University of Texas study also progressed to bulbar involvement.58 Another series has provided the most detailed information regarding timing of progression: in 14 of 25 patients, labeled the ascending group, symptoms spread to the second leg an average of 1.7 years later (range 1–4 years), to the hands 3.6 years later (range 1–6 years), and to the bulbar region 1.5 years (range 0.5–5 years) after arm involvement.75
Some investigators have described cases progressing directly from bulbar regions to the leg, or from the leg to bulbar regions, without significant arm involvement. Even in these cases, however, brisk reflexes and other upper motor neuron signs were noted in the upper limbs. Conversely, some patients experienced periods of symptomatic plateau with only one region affected. Russo described four patients with spastic paraparesis without significant progression over 2–3.5 years, and Sotaniemi and Myllyla reported two patients with spastic paraparesis and upper extremity hyperreflexia remaining stable during 2–3 years of follow-up.

With lower-limb onset, symptoms usually begin unilaterally. Spasticity produces most of the limb dysfunction in early stages, unlike ALS in which weakness usually predominates. PLs patients typically report stiffness, clumsiness, or poor coordination as the initial limb symptom; when limb weakness occurs, it generally follows an upper motor neuron pattern.

Bulbar symptoms usually manifest first as dysarthria, followed by dysphagia, and may evolve to emotional lability and inappropriate laughing or crying (pseudobulbar affect). Dysarthria can progress to anarthria. Worsening dysphagia may necessitate feeding-tube placement, even in the absence of significant limb involvement. Emotional lability was seen in 5 of 10 patients in one series, and in 17 of 20 described in another. Similarly, Zhai et al. noted it as a “frequent later symptom.”

Although Erb’s triad of spasticity, hyperreflexia, and mild weakness remain as the most prominent manifestations of PLs, several other features have been noted consistently in PLs patients. These features include eye movement abnormalities, urinary dysfunction, and cognitive impairment.

Patients do not complain of visual symptoms, but abnormalities of eye movements may be noted on examination. Pringle et al. noted saccadic breakdown of smooth pursuit in seven of eight patients. Similarly Le Forestier et al. described progressive supranuclear paralysis in 2 of their 20 patients.

Urinary complaints were initially considered to indicate a different disease process. The Pringle criteria describe preservation of bladder function as additionally suggestive of PLs; still, they noted that half their patients developed bladder symptoms in later stages of disease. Other series have reported urinary urgency or incontinence as a frequent symptom, usually developing several years after initial presentation (Table 1). Such symptoms appear more commonly in PLs than ALS, likely due to detrusor hyperreflexia or spasticity of the internal urinary sphincter.

In most early studies, cognition was reported as normal. Le Forestier et al. first noted that 16 of 20 patients demonstrated abnormalities when tested for frontal lobe function, although dementia was not observed. Others have reported a frontal lobe dementia syndrome in PLs patients, and Gordon et al. noted that 2 of 34 patients demonstrated cognitive impairment. These observations, consistent with the finding of frontal lobe dementia in 10–20% of ALS patients, have contributed to the increasing awareness noted earlier that motor neuron disease may reflect one feature of a more general neurodegenerative process.

**Disease Course and Prognosis**

PLs tends to follow a very slowly progressive course, a key distinctive clinical feature compared with ALS. Most reports indicated that patients were still alive, whereas the average life expectancy for patients with ALS is about 3 years. Longevity data for PLs are incomplete. Recent series documented duration of symptoms at the time that patients were either diagnosed or reported in the literature; seven studies described an average disease duration of 7.9 years or longer (Table 1). Among PLs patients with reported deaths, survival ranged from 1 to 15 years after onset but, of note, none of the deaths were directly attributable to PLs.

Periods of clinical stability lasting months were observed in half of the patients in one series. Follow-up on 20 patients showed 5 could walk unaided, 5 used a walker, and 10 needed a wheelchair after 4–6 years.

There are reports of patients initially thought to have PLs, but subsequently diagnosed with ALS. In an early series, Spiller reported that, on autopsy, six of eight patients diagnosed with PLs while alive had histologic evidence favoring ALS. More recently, Bruyn et al. described three patients displaying exclusively upper motor neuron clinical symptoms for 7.5, 9, and 27 years, until the development of fasciculations, atrophy, and EMG findings prompted revision of the diagnosis to ALS. Similarly, Le Forestier et al. found that 3 of 20 patients, after disease durations of 11–13 years, eventually satisfied criteria for probable ALS.

Gordon et al. identified a group of 29 patients with isolated upper motor neuron signs and no evidence of lower motor neuron involvement clinically or on EMG. Of that group, 13 went on to develop EMG evidence of denervation, as well as clinical
signs of lower motor neuron dysfunction. These investigators therefore proposed a more detailed set of diagnostic categories for the PLS-spectrum disorders (Table 2).

For patients with a diagnosis of PLS and minimal EMG changes, there seems to be more disability compared to PLS patients without EMG changes. Singer et al. reported a significantly decreased ability to ambulate independently in their patients with PLS and minimal EMG changes, even after controlling for increased duration of illness in that group.58 Gordon et al. noted significant differences in scores on the ALS Functional Rating Scale–Revised between their clinically pure PLS group versus the upper motor neuron (UMN)-dominant ALS group, with a trend toward lower forced vital capacity and reduced time to disability in the UMN-dominant ALS group.29

Overall, the available data indicate a more benign prognosis for PLS than for ALS. The import of making a clinical distinction is readily apparent when managing and counseling patients and their families, even if a nosologic determination remains unclear.

**LABORATORY FINDINGS**

Studies recommended to exclude other diagnoses (Table 3) include normal serum chemistries and vitamin B12 levels; testing for syphilis, Lyme disease, and human T-cell lymphototrophic virus-1; examination of cerebrospinal fluid; and magnetic resonance imaging to exclude compressive lesions or hyperintensities suggestive of multiple sclerosis.55 Currently, many investigators also obtain serum long-chain fatty acids to exclude adrenomyeloneuropathy, and HIV serologies in patients at risk. All patients should undergo EMG and nerve conduction studies. Further testing, such as evoked potentials and muscle biopsy, should be performed according to the clinical situation. For symptom onset primarily in the legs, testing for genetic mutations associated with hereditary spastic paraparesis (SPG3A, SPG4, and SPG6) is now commercially available. Significantly, Brugman et al. screened for mutation of the SPG4 (spastin) gene, the most common cause of hereditary spastic paraparesis, in a population of 99 patients with adult-onset UMN symptoms.8 With the exception of one patient later determined to have ALS, no such mutations were identified in patients with symptoms in the arms or bulbar region. Of the subgroup of 47 patients with symptoms exclusively in the legs, a mutation was identified in 13%.

**Creatine Kinase.** Elevated serum creatine kinase (CK) levels in motor neuron disease reflect muscle injury from lower motor neuron degeneration. When examined in patients with ALS, approximately 40% were found to have elevated levels.20,35,43 Serum CK results are not reported frequently in PLS series. In one study, only 3 of 18 patients tested (17%) had elevated levels; 2 of the 3 had EMG evidence of denervation.58 Kuipers-Upmeijer et al. found elevated serum CK levels in 4 of 10 patients, 2 of whom had denervation changes on EMG.40

**Electromyography.** Although the PLS diagnostic criteria proposed by Pringle et al. (Table 3) exclude clinical signs of lower motor neuron (LMN) dysfunction, these guidelines do allow for electrophysiologic evidence of mild denervation, manifest as “occa-

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**Table 2. Diagnostic categories of PLS suggested by Gordon et al.**

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<tr>
<th>Category</th>
<th>Definition</th>
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<tr>
<td>Autopsy-proven PLS</td>
<td>Clinically diagnosed PLS with degeneration in motor cortex and corticospinal tracts, no loss of motor neurons, no gliosis in anterior horn cells, and no Bunina bodies or ubiquinated inclusions.</td>
</tr>
<tr>
<td>Clinically pure PLS</td>
<td>Evident upper motor neuron signs, no focal muscle atrophy or visible fasciculations, and no evidence of denervation on EMG at ≥4 years from symptom onset. Age at onset after 40. Secondary and mimicking conditions excluded by laboratory and neuroimaging.</td>
</tr>
<tr>
<td>UMN-dominant ALS</td>
<td>Symptoms &lt;4 years, or disability due predominately to UMN signs but with minor EMG denervation or LMN signs on examination that are not sufficient to meet diagnostic criteria for ALS.</td>
</tr>
<tr>
<td>PLS plus</td>
<td>Predominant UMN signs plus clinical, laboratory, or pathologic evidence of dementia, parkinsonism, or sensory tract abnormalities. (If cerebellar signs, urinary incontinence, or orthostatic hypotension are evident, multiple-system atrophy should be considered.)</td>
</tr>
<tr>
<td>Symptomatic lateral sclerosis</td>
<td>Clinically diagnosed PLS with evident possible cause (e.g., HIV infection, paraneoplastic syndrome).</td>
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UMN, upper motor neuron; MND, motor neuron disease; PLS, primary lateral sclerosis.
sional fibrillation and increased insertional activity in a few muscles (late and minor).”

Several series have based the diagnosis of PLS at least in part on the presence of normal electrodiagnostic results.2,26,27,29,57 In one series, no EMG findings of lower motor neuron involvement were encountered among 25 patients in the prior 3 years.75 In other larger series, however, subtle but definite abnormalities on needle EMG have been noted (Table 1). Younger et al. described seven cases separately because of evidence of denervation on EMG.74 These patients were considered to have “PLS with EMG denervation”; on follow-up, one developed clinical evidence of lower motor neuron disease, and was subsequently diagnosed with ALS. As noted previously, Gordon et al.29 categorized patients displaying EMG denervation abnormalities as “UMN-dominant ALS,” and noted in their patients that such findings were ultimately followed by development of LMN clinical signs. Singer et al. reported 10 of 25 patients with features of mild active denervation in one or more muscles (increased insertional activity, grade 1+ fibrillations or positive sharp waves) or fasciculations; decreased recruitment of motor units was seen, but motor unit morphology was normal.58 None of these patients had sufficient abnormalities on EMG for diagnosis of ALS based on the revised El Escorial criteria.5 Of note, the patients with EMG features of denervation developed disease at a later age, but progressed more rapidly.56 In the Le Fores-tier et al. study, evidence of active or chronic denervation was observed in 95% of patients at some stage of illness.51 Fibrillation potentials were seen in early stages of disease or as a transient feature on repeat studies. The significance of their disappearance is not clear, and may reflect successful reinnervation or simply variation in sampling.

A pure UMN syndrome in two regions can fulfill the El Escorial research criteria, as originally proposed in 1994, for clinically suspected ALS.4 Identification of even minimal EMG abnormalities in two limbs to the degree reported in some PLS publications would meet the revised El Escorial criteria (2000) for clinically probable laboratory-supported ALS.5 Gordon et al.29 noted that clinical LMN signs invariably follow EMG features of denervation, although this observation has not been consistent across other series.40,41,58,74

Table 3. Proposed PLS diagnostic criteria by Pringle et al.53

<table>
<thead>
<tr>
<th>Clinical</th>
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<tbody>
<tr>
<td>Insidious onset of spastic paresis, usually beginning in the lower extremities, but occasionally bulbar or in an upper extremity.</td>
</tr>
<tr>
<td>Adult onset; usually fifth decade or later.</td>
</tr>
<tr>
<td>Absence of family history.</td>
</tr>
<tr>
<td>Gradually progressive course.</td>
</tr>
<tr>
<td>Duration ≥3 years.</td>
</tr>
<tr>
<td>Clinical findings limited to those usually associated with corticospinal dysfunction.</td>
</tr>
<tr>
<td>Symmetric distribution, ultimately developing severe spastic spinobulbar paresis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory studies to help exclude other diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum chemistries, including normal vitamin B₁₂ levels.</td>
</tr>
<tr>
<td>Negative serologic tests for syphilis; in endemic areas, negative Lyme and HTLV-1 serologies.</td>
</tr>
<tr>
<td>Normal cerebrospinal fluid parameters, including absence of oligoclonal bands.</td>
</tr>
<tr>
<td>Absent denervation potentials on EMG or at most, occasional fibrillation and increased insertional activity in a few muscles (late and minor).</td>
</tr>
<tr>
<td>Absence of high-signal lesions on MRI similar to those seen in multiple sclerosis.</td>
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</table>

<table>
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<tr>
<th>Additionally suggestive of PLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preserved bladder function.</td>
</tr>
<tr>
<td>Absent or very prolonged latency on cortical motor evoked responses in the presence of normal peripheral stimulus-evoked maximal compound muscle action potentials.</td>
</tr>
<tr>
<td>Focal atrophy of precentral gyrus on MRI.</td>
</tr>
<tr>
<td>Decreased glucose consumption in pericentral region on PET scan.</td>
</tr>
</tbody>
</table>

PLS, primary lateral sclerosis; HTLV-1, human T-cell lymphocytotropic virus-1; MRI, magnetic resonance imaging; PET, positron emission tomography.

OTHER TESTING

Motor Evoked Potentials. Several large series have noted abnormalities in motor evoked potentials (MEPs) in PLS patients, unlike in ALS, where central motor conduction time is typically normal or only mildly delayed.40 Brown et al. observed that MEPs were absent in the legs and arms in four of seven cases; when MEPs were elicited, central motor con-
duction time was prolonged to two to three times the upper limit of normal.6 Le Forestier et al. could not obtain responses in 12 of 20 patients in either the upper or lower limbs.41 Similar MEP abnormalities were seen in two other PLS series.40,53 Zhai et al.75 found that in the ascending group all 13 patients had absent MEPs in hand muscles, but in the multifocal group, 6 of the 7 patients had normal central motor conduction times.

Sensory Evoked Potentials. Although patients with PLS do not manifest clinical sensory deficits, some data suggest that subtle abnormalities are indeed present. Recent series have noted abnormal sensory evoked potentials. Le Forestier et al. observed prolonged visual, median, or tibial evoked potentials in 11 of 20 patients.41 Kuipers-Upmeijer et al. found prolonged latencies on median and peroneal sensory evoked potentials in several patients with intact sensation.40

Motor Neuron Firing. Floeter et al. inserted intra-muscular wire electrodes into wrist extensor muscles of nine PLS patients and seven controls to measure responses to voluntary contraction, as well as activation of muscle spindle 1a afferents by vibration.24 Normally, motor neurons increase their firing rate with increasing force, and then recruit larger motor units to create a smoothly gradated pattern. These investigators noted, however, that motor units in PLS patients did not increase firing rates with increasing force. With voluntary contraction or vibration, the change of firing frequency with increasing excitatory input was flat or slightly decreased, a finding similar to the alteration in motor neuron firing observed in patients with spasticity due to stroke. The investigators hypothesized that abnormal activation of voltage-gated channels, abnormal recruitment of motor neuron pools, or tonic inhibition of motor neurons could account for these results. Such alterations likely contribute to the sense of weakness and early fatigue in PLS.

Neuroimaging. Routine computed tomography and magnetic resonance brain imaging in PLS is typically normal or shows only nonspecific changes. Of note, frontal lobe atrophy has been reported in some series, most consistently by Pringle et al., where atrophy of the precentral gyrus was seen in six of eight patients.59 Smith presented a case report of serial magnetic resonance imaging performed over 9 years.50 The imaging showed progressive atrophy of the premotor, parietal, and primary sensorimotor cortex, with sparing of the temporal and occipital lobes and cerebellum.

Chan et al.14 examined magnetic resonance spectroscopy of the motor cortex in 18 patients diagnosed with PLS, noting a reduced N-acetylaspartate/creatine ratio in 12 cases. Zhai et al.75 reported results for nine patients in the ascending group. Mean N-acetylaspartate/creatine and N-acetylaspartate/choline ratios in the motor cortex were reduced compared to controls, suggesting atrophy, dysfunction, or loss of neurons in the motor cortex. Similar findings have been reported in patients with ALS.14,52,65

Positron emission tomography in PLS patients has demonstrated abnormalities in the precentral gyrus region, including regional decrease in fluorodeoxyglucose uptake and cerebral blood flow, and a reduction in regional density of benzodiazepine receptors (a putative index of cortical neuronal density).42,53 These findings would not distinguish PLS from ALS.

More recently, diffusion tensor imaging has demonstrated decreased image intensities of the posterior limb of the internal capsule compared to controls, reflecting decreased diffusion anisotropy and implying damage to or dysfunction of these pathways.69 Such changes can be seen in patients with PLS as well as ALS, and may serve as a tool to grade upper motor neuron involvement in both diseases.15

Muscle Biopsy. Muscle biopsies are only rarely performed in PLS. An exception is the Le Forestier et al. series, in which the deltoid was examined in all 20 patients.41 Signs of either denervation or reinnervation were seen in 13 samples, and signs of both were present in 11. They contended that denervation may be present throughout the course of the disease, consistent with their observation of early EMG abnormalities. Singer et al. described biopsy results in four patients from their series; the histology demonstrated minimal denervation atrophy with rare angulated fibers.58

Autopsy. Only a small number of postmortem analyses have been reported for patients with PLS. In the pioneering work by Erb,18 Spiller,61 and Buzzard,11 the most common pathologic finding was degeneration of the corticospinal tracts, with mild degeneration of the cerebellar tracts and fasciculus gracilis. Conclusions from this earlier literature are of uncertain interpretation, however, because of the limited ability at that time to exclude other diagnoses. Six autopsies in the modern era (Table 4) have reported a common finding of loss of myelinated fibers
throughout the corticospinal pathway, with sparing of anterior-horn neurons. Most also displayed atrophy of the precentral gyrus and loss of Betz cells in the motor cortex. As noted by Rowland, however, these autopsy reports also must be interpreted with caution, because they were obtained prior to widespread recognition of Bunina bodies and ubiquitinated neuronal inclusions as key features of the pathology of ALS.56 There have been six published autopsies since 1997 of patients with UMN-dominant symptoms (Table 5).36,38,50,56,64,68,70 Further pathologic studies and clinicopathologic correlations are needed to better relate the spectrum of symptoms with underlying pathologic alterations.

Differential Diagnosis

The differential diagnosis for progressive corticospinal spasticity, alone or together with corticobulbar spasticity, is broad.58 Many disorders, such as inherited illnesses or conditions with sensory symptoms or more generalized neurologic impairment, could be eliminated readily after a careful history and examination. Although PLS generally has been a diagnosis of exclusion, the same consideration applies to most sporadic degenerative conditions for which no diagnostic test is available. In reality, if a patient presents with slowly progressive spinobulbar spasticity in the absence of other findings, the differential diagnosis is limited. The hereditary spastic paraplegias (HSP) and familial ALS merit particular consideration.

The inherited forms of spasticity were originally termed hereditary PLS by Erb, and later referred to as Strumpell’s disease (an autosomal-dominant form of HSP now designated spastic paraplegia type 3).3,34 The last decade has witnessed rapid progress in clarifying the genetic basis for several forms of HSP. HSP can be autosomal dominant, recessive, or X-linked. Twenty genetic loci (spastic gait loci, SPG) have been identified thus far.21,22 As noted previously, the most common form, accounting for 40% of autosomal-dominant cases, is associated with mutation of the SPG4 gene, which produces the protein spastin. Other autosomal-dominant forms are caused by mutations of the genes encoding atlastin (SPG3), kinesin heavy chain (SPG10), NIPA1 (SPG6), and heat shock protein 60 (SPG13). Autosomal-recessive inheritance has been linked to mutations in paraplegin (SPG7) and spartin (SPG20), and X-linked forms to L1 cell adhesion molecule (SPG1) and proteolipid protein (SPG2). Currently, genetic testing is commercially available for spastin, atlastin, and NIPA1 mutations.21

HSP usually presents prior to 20–30 years of age, most commonly in adolescence. There is some range in the age of onset of illness, including patients diagnosed in middle age with symmetric leg spasticity. Although lower-extremity spasticity generally progresses in severity, arm involvement is uncommon, and bulbar manifestations are extremely rare.48 Interestingly, lower motor neuron dysfunction has been reported in a subgroup of patients with mutation of SPG4.49 Bladder symptoms are com-

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Onset (years)</th>
<th>Gender</th>
<th>Duration (years)</th>
<th>Symptom onset</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>67</td>
<td>M</td>
<td>5</td>
<td>LE</td>
<td>Betz cells in the precentral gyrus “probably” decreased; degeneration of corticospinal tract from medullary pyramids throughout the spinal cord.</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>F</td>
<td>3</td>
<td>B</td>
<td>Bilateral atrophy of precentral gyrus, with loss of Betz cells and marked gliosis; degeneration of corticospinal tract from internal capsule through the spinal cord. Intracytoplasmic eosinophilic inclusion bodies in the hypoglossal nucleus and anterior-horn neurons in the cervical and lumbar regions.</td>
</tr>
<tr>
<td>74</td>
<td>71</td>
<td>M</td>
<td>6</td>
<td>LE</td>
<td>Degeneration of corticospinal tract from the cerebral peduncles through all levels of the spinal cord, with no loss of Betz cells or LMNs. Adenocarcinoma of the lungs with metastases.</td>
</tr>
<tr>
<td>74</td>
<td>66</td>
<td>M</td>
<td>11</td>
<td>LE</td>
<td>Degeneration of corticospinal tract from medulla through all levels of the spinal cord; no loss of Betz cells or LMNs. Arthritic ridge at C6–7; cystic areas of encephalomacia in right caudate, thalamus, and frontal gyrus.</td>
</tr>
<tr>
<td>74</td>
<td>60</td>
<td>M</td>
<td>1</td>
<td>LE</td>
<td>Degeneration of corticospinal tract from internal capsule through all levels of the spinal cord; no loss of Betz cells or LMNs. EMG showed 1–2+ fasciculations.</td>
</tr>
<tr>
<td>53</td>
<td>71</td>
<td>M</td>
<td>15</td>
<td>LE</td>
<td>Loss of Betz cells in the precentral gyrus; degeneration of corticospinal tract from internal capsule through all levels of the spinal cord, with gliosis of the anterior horn but no loss of LMNs. EMG showed occasional fibrillation potentials.</td>
</tr>
</tbody>
</table>

M, male; F, female; LE, lower extremities; B, bulbar; LMN, lower motor neuron.
mon, and some patients may exhibit mild sensory loss in the feet, suggesting posterior column involvement. It is possible that some reported patients with PLS actually had a form of HSP, although the absence of family history, typical onset in middle age or later life, and bulbar involvement would make this prospect less likely.8

In familial ALS, patients usually display overt upper and lower motor neuron involvement. Rare cases of PLS have been reported, however, in families with non–superoxide dismutase 1 autosomal-dominant ALS9 and SMA-4.1 Autosomal-recessive juvenile-onset forms of ALS have been identified, related to mutations in the ALS2 gene, a gene coding for the GTPase alsin.33,39 Of considerable interest, mutations in this same gene have been associated with autosomal-recessive cases of infantile ascending HSP and juvenile PLS.19,39,51,56 It remains unclear how mutations in the same gene can lead to three very different phenotypes; the age of onset, however, is usually in the first 2 years of life, unlike typical PLS.31,73

For patients with exclusive upper motor neuron symptoms acquired in middle age or later, the two main diseases to consider are ALS and PLS. ALS is more common, and is usually the ultimate diagnosis. Stark and Moersch suggested 5 years as the minimal duration of symptoms before diagnosing PLS.62 Pringle et al. proposed 3 years, if additional criteria were also met (Table 3).53 Gordon et al., however, observed that, of their patients who developed lower motor neuron signs, 46% did so by 3 years and 77% by 4 years.29

If there is no or very limited evidence of lower motor neuron involvement, a tentative diagnosis of a PLS-spectrum disorder can be made (see later). In this setting, patients can be informed that the prognosis is better compared to ALS, and that the progression is slow. Such patients require close follow-up to determine whether lower motor neuron signs develop.

Reports of Concurrent Diseases. As mentioned previously, several reports exist of patients with PLS and

<table>
<thead>
<tr>
<th>Reference no.</th>
<th>Onset (years)</th>
<th>Gender</th>
<th>Duration (years)</th>
<th>Clinical syndrome</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>63</td>
<td>M</td>
<td>3</td>
<td>UMN findings; DA; DP</td>
<td>Clinically suspected PLS. Betz cells absent; CST degeneration; no loss of anterior-horn cells; hyaline inclusions without Bunina bodies in cervical MNs; ubiquitin-positive inclusions in cervical and lumbar MNs.</td>
</tr>
<tr>
<td>38</td>
<td>76</td>
<td>M</td>
<td>2.75</td>
<td>UMN findings; parkinsonian features; anarthric; DP; occasional fasciculations</td>
<td>Complicated PLS, described as UMN-predominant degeneration with FT atrophy; Betz cells absent; ubiquinated inclusions without Bunina bodies in precentral, cingulate, and temporal cortex; CST degeneration; no loss of anterior horn cells, but occasional ubiquinated skeins and few Bunina bodies.</td>
</tr>
<tr>
<td>64</td>
<td>38</td>
<td>F</td>
<td>17</td>
<td>UMN findings; DA; DP; pseudobulbar palsy; dementia</td>
<td>Complicated PLS with dementia. Loss of pyramidal cells in precentral gyrus; CST degeneration; anterior horn cells relatively well-preserved, with no Bunina bodies or ubiquinated inclusions; “ballooned” structures in Purkinje and molecular layer of cerebellum.</td>
</tr>
<tr>
<td>68</td>
<td>75</td>
<td>F</td>
<td>7.3</td>
<td>UMN findings; dementia; anarthic</td>
<td>Complicated PLS with dementia. CST degeneration; few Bunina bodies and ubiquinated skeins in anterior horn; FT lobar degeneration with ubiquinated inclusions.</td>
</tr>
<tr>
<td>36</td>
<td>50</td>
<td>F</td>
<td>6</td>
<td>UMN findings; dementia</td>
<td>Investigators describe case as sporadic MN disease with FT atrophy mimicking PLS. CST degeneration; no loss of anterior-horn cells, but ubiquitin-positive inclusions; neuronal loss in hypoglossal nucleus.</td>
</tr>
<tr>
<td>50</td>
<td>57</td>
<td>M</td>
<td>9</td>
<td>Pseudobulbar palsy; anarthria; DP; parkinsonian features</td>
<td>Complicated PLS with severe FT atrophy and parkinsonian features. Loss of pigment in substantia nigra; atrophy of cerebral peduncles and pyramids; Betz cells absent; CST degeneration; ubiquitin-positive inclusions in the FT cortex and basal ganglia; no Bunina bodies; no loss of anterior-horn cells reported.</td>
</tr>
</tbody>
</table>

CST, corticospinal tract; DA, dysarthria; DP, dysphagia; F, female; FT, frontotemporal; M, male; MN, motor neuron; NR, not reported; PLS, primary lateral sclerosis; UMN, upper motor neuron.
concurrent medical diagnoses. Five women were reported with breast cancer and PLS; three were ultimately diagnosed with ALS. An autopsy report described a patient with multiple myeloma and an upper motor neuron syndrome in the absence of cord infiltration by tumor, and a PLS case of 7-year duration was reported in association with an IgM paraprotein. Two HIV-infected patients, one of whom developed full acquired immune deficiency syndrome, were described with symptoms of PLS. These cases raise the possibility, as discussed by Rowland, that PLS may represent a syndrome of diverse etiology.

**DIAGNOSTIC CRITERIA**

In 1945, Stark and Moersch proposed diagnostic criteria that remained in common use until 1992, when they were superseded by those of Pringle et al. that included modern testing. The Pringle criteria (Table 3) require symmetric symptom distribution, suggest preserved bladder function, and do not distinguish between patients with minimal EMG evidence of denervation and those with no such changes on EMG.

Brugman et al. described PLS patients who had a family history of ALS, and recommended that the diagnostic criteria be modified to permit a family history of MND where appropriate. It is more likely, however, that these patients are HSP cases in which the specific mutation has not been identified.

As mentioned earlier, Gordon et al. noted that development of lower motor neuron signs inevitably follow EMG findings of denervation, and in 77% of their cases occurred within 4 years of symptom onset. They therefore argued that a 4-year span be a component of the diagnostic criteria, and proposed more specific nomenclature based on the constellation of clinical and EMG features (Table 2).

These valuable insights are incorporated into an integrated set of diagnostic guidelines for the PLS-spectrum disorders (Table 6), combining clinical and laboratory features to classify patients as clinical PLS, suspected PLS, or complicated PLS (PLS plus).

**TREATMENT**

There remains no cure for PLS. Unlike ALS, no studies have shown that riluzole slows progression of disease in PLS. As in the treatment of ALS, patients can be placed on high-dose antioxidant vitamins C and E, and betacarotene, although no rigorous clinical data are available to support this approach.

Treatment is symptom directed. For spasticity, baclofen or tizanidine are first-line agents. Dantrolene is infrequently used, particularly since develop-

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**Table 6. Diagnostic criteria for PLS**

**Clinical features**

Presence of:

1. Findings of UMN disorder on examination:
   - Spasticity
   - Pathologic reflexes
   - Weakness, when present, in UMN distribution
2. Regions of involvement:
   - Limb (legs, arms, or both)
   - Bulbar
   - Mixed limb and bulbar
3. Time course ≥4 years
4. Progressive
5. Age of onset: adult >20 years
6. Bladder symptoms due to UMN dysfunction may be present

Absence of:

1. LMN disorder on examination:
   - Fasciculations
   - Atrophy
2. Sensory signs on examination
3. Family history of similar disorder

**Laboratory features**

Supportive of:

1. Transcranial magnetic stimulation:
   - Increased CMCT
   - Decreased cortical excitability (e.g., MEP cannot be obtained)
2. Magnetic resonance spectroscopy findings in motor cortex
   - Decreased NAA/Cr ratio
   - Decreased NAA/Cho ratio
3. Diffusion tensor imaging findings in posterior limb of internal capsule
   - Decreased FA
   - Increased ADC

Exclusionary:

1. Serologic evidence for etiology of myelopathy
2. Abnormal CSF
3. Evidence of structural abnormality or demyelinating disorder on routine MRI of brain or spine
4. EMG:
   - Abnormal motor or sensory NCS
   - See classification section (below) for patients with EMG abnormalities
5. Any known HSP or alsin gene mutations

**Classification of PLS**

**Clinical PLS:**

- Fulfills above clinical and laboratory inclusionary and exclusionary features

**Suspected PLS:**

- Fulfills above clinical and laboratory inclusionary and exclusionary features except:
  1. ≤4-year duration
  2. EMG evidence of minimal denervation that does not satisfy El Escorial criteria for ALS

**Complicated PLS (PLS plus):**

- Patients fulfill criteria for clinical PLS or suspected PLS but also have evidence of dementia, parkinsonism, or sensory abnormalities

ADC, apparent diffusion coefficient; Cho, choline; CMCT, central motor conduction time; Cr, creatinine; CSF, cerebrospinal fluid; EMG, electromyography; FA, fractional anisotropy; HSP, hereditary spastic paraparesis; LMN, lower motor neuron; MEP, motor evoked potential; NAA, N-acetylaspartate; NCS, nerve conduction study; PLS, primary lateral sclerosis; UMN, upper motor neuron.
velopment of baclofen pump technology provides a useful alternative in refractory cases. Zhai et al. noted that 12 of 25 patients were on baclofen or tizanidine, 2 patients obtained baclofen pumps during follow-up, and an additional 4 patients were using benzodiazepines. Tricyclic antidepressants such as amitriptyline can help control excessive oral secretions, as well as pseudobulbar symptoms of emotional incontinence. Dooring may also respond to other anticholinergic medications, such as hyoscymine, benztprine mesylate, glycopyrrolate, or scopolamine patches; preliminary data indicate that injection of botulinum toxin into the submandibular glands decreases saliva production in ALS patients.

Overall, it is preferable for patients to be managed in a multidisciplinary motor neuron disease clinic, so that various issues, including speech, pulmonary function, nutrition, social work, counseling, and physical and occupational therapy, can be addressed in a comprehensive and cooperative manner.

REFERENCES


ABSTRACT: Mechanical deformation of a peripheral nerve can evoke action potentials in sensory and motor axons. The generation of these impulses with brief stimuli (<0.5 s) and their relationship to the deformation conditions have not been systematically studied in human subjects. Controlled compression stimuli over a range of amplitudes, durations, and loading rates were delivered to the ulnar nerve at the medial epicondyle in awake human subjects. Compound muscle action potentials were recorded from the first dorsal interosseous muscle. Subjects rated the magnitude of evoked paresthesias. Mechanically evoked motor and sensory responses varied linearly with the magnitude (P < 0.001) and rate of deformation (P < 0.01), but not the duration, and occurred only during the compression phase. Cutaneous axons had lower mechanical thresholds than motor axons. We relate these findings to the viscoelastic properties of peripheral nerves and differences in biophysical properties of cutaneous and motor axons.


MECHANICALLY EVOKED SENSORY AND MOTOR RESPONSES TO DYNAMIC COMPRESSION OF THE ULNAR NERVE

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All nerve fibers exhibit mechanosensitivity, in which an adequate mechanical stimulus can evoke action potentials at a site remote from the normal site of initiation (i.e., the cell body or sensory terminals). Such ectopically generated action potentials can be evoked by, for example, brisk compression of the ulnar nerve against the medial epicondyle of the elbow (‘funny bone’), or by percussion of the median nerve at the wrist in cases of carpal tunnel syndrome (Tinel’s sign). The generation of ectopic impulses by cutaneous axons is perceived as paresthesias, whereas those generated by motor axons produce fasciculation. There has been no systematic study of the relative susceptibilities of cutaneous and motor axons to compressive mechanical stimuli delivered at a point along the length of human axons in situ. Accordingly, the underlying mechanisms for mechanically evoked paresthesias and fasciculation are poorly understood.

Chronic compression is a common type of peripheral nerve injury that can arise through entrapment neuropathies such as carpal tunnel syndrome (CTS) or constant prolonged external pressure, such as Saturday night palsy. Mechanical deformation via dislocation of the myelin around the node of Ranvier is thought to be the primary mechanism of the latter,23 whereas ischemia has been implicated in idiopathic CTS.12,15,27 Studying the effects of acute nerve compression may provide insights into the mechanisms underlying chronic nerve compression. Various studies of the neural responses to mechanical loading in animal models have focused primarily on morphological or conductance changes, or on identifying the loading thresholds at which electrophysiological dysfunction occurs in vivo with both stretch8,10 and compression.6,25 A single-axon in vitro model demonstrated loading rate-related changes in membrane potential and its recovery time course that were reversible for small stretches (<20%), whereas larger stretches produced nonre-

Abbreviations: ADM, abductor digiti minimi; ANOVA, analysis of variance; CMAP, compound muscle action potential; CTS, carpal tunnel syndrome; FDI, first dorsal interosseous; LVDT, linear variable differential transformer; SNAP, sensory nerve action potential; SNK, Student–Newman–Keuls

Key words: axonal excitability; carpal tunnel syndrome; mechanical injury; mechanotransduction; peripheral nerve

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versible injury. Similarly, a rate- and magnitude-dependent response to stretch has also been observed in a population of cultured cerebral cortical cells in vitro. Acute compression (<10 s) of neural tissue evokes only brief responses (<10 s) in spinal dorsal roots, but ongoing activity (lasting several minutes) in the dorsal root ganglia. Furthermore, acute stretch (~1 s) of isolated sensory nerve fibers evokes responses that continue for as long as the stretch is applied.

We investigated the effects of controlled acute nerve compression in awake human subjects. We varied the loading rate, amplitude, and duration of dynamic mechanical stimuli applied perpendicular to the nerve and compared the results of mechanically evoked discharges in sensory (cutaneous) and motor axons. Our results suggest that sensory axons have lower thresholds to mechanical deformation than motor axons and highlight some of the changes in the nerve that precede ischemia and injury-related changes in connective tissue density. Some of this work has been presented in abstract form.

MATERIALS AND METHODS

Subjects. Twelve experiments were performed on eight healthy, neurologically normal subjects (five women, three men, age 20–27 years). All subjects gave informed consent and approval was given by our institutional review board. Subjects lay prone with the shoulder and elbow on the test side flexed to 90°. The hand was stabilized on a solid platform with the wrist at 180° and the digits immobilized in a handle grip position (Fig. 1A). Subjects were instructed to relax and be as still as possible for the duration of the experiment. The ulnar nerve was located by electrical stimulation over the groove between the medial epicondyle and the olecranon (2–10 mA, 0.2 ms, 1 Hz; Stimulus Isolator, ADInstruments, Sydney, Australia) and observation of the twitches evoked in the intrinsic muscles of the hand and the subject’s reports of paresthesias. The site of optimal response was marked. The depth of maximum possible indentation at this point was measured using a blunt probe.

Mechanical Stimulation. A custom-made blunt cylindrical probe (6, 7, or 8 mm in diameter, with the size chosen according to the width of the groove between the medial epicondyle and the olecranon) was used to deliver controlled compression orthogonal to the ulnar nerve. The probe was attached to a linear motor (Baldor, LMM2-1F5-1F1, Fort Smith, Arkansas). The extent of the motor’s excursion was servo-controlled via a custom-made proportional integral differential controller. Feedback control of probe position was provided by a linear variable differential transformer (LVDT) in series with the shaft of the motor. The motor command voltage and LVDT signals were recorded (PowerLab 16S, ADInstruments) with a sampling frequency of 200 Hz.

The probe was aligned perpendicular to, and just contacting, the subject’s skin at the marked location of the ulnar nerve. Subjects were familiarized with the range of mechanical stimuli prior to testing. Stimulus conditions were presented in random order using step and triangular waveform profiles (Fig. 1B), each at three different amplitudes. Due to anatomical differences between subjects, the mechanical stimulus amplitudes could not be the same for each subject. The maximum amplitude was equivalent to the measured maximum indentation at the

FIGURE 1. Experimental design. (A) Typical experimental arrangement. (B) Typical stimulus profiles: triangular profiles (i, ii) were delivered over three amplitudes (maximum compression, maximum minus 2 mm, maximum minus 4 mm) at five frequencies (2, 4, 6, 8, and 10 Hz). Step profiles (iii, iv) were delivered over the same amplitudes at five durations (0.5, 0.25, 0.17, 0.13, 0.1 s).
site of mechanical stimulation, the medium amplitude was calculated as the maximum minus 2 mm, and the minimum amplitude was calculated as maximum minus 4 mm. Thus, stimulus amplitudes varied by a constant displacement, rather than percentage of maximum compression. The displacement amplitudes for the smallest subject were 2, 4, and 6 mm, whereas the largest subject required 8, 10, and 12 mm. Triangular stimuli were delivered at five frequencies (2, 4, 6, 8, and 10 Hz). Step stimuli were delivered for five durations (0.5, 0.25, 0.167, 0.133, 0.1 s). Each stimulus condition was presented as a set of 10 stimuli with a 2-s interstimulus interval. Each condition was presented in a random order separated by a 60-s delay.

**Compound Muscle Action Potential Recording.** Compound muscle action potentials (CMAPs) were recorded from the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles using disposable 10-mm Ag/AgCl surface electrodes. The signal was recorded at a sampling rate of 2 kHz with a gain of 5,000, bandpass filtered from 10 Hz to 1 kHz, and notch filtered at 50 Hz (BioAmplifier, PowerLab, ADInstruments). CMAP peak-to-peak amplitudes, CMAP onset latency, and CMAP peak latency were measured for each stimulus and were averaged for each series of 10 mechanical stimuli using Chart software (ADInstruments).

**Sensory Responses.** Immediately after each set of 10 stimuli, subjects were asked to report an average intensity of paresthesias using a modified Borg scale, in which 0 represented no sensation and 10 represented the maximum possible sensation. Subjects were instructed to base this scale on trial stimuli presented for five durations (0.5, 0.25, 0.167, 0.133, 0.1 s). Each stimulus condition was presented as a set of 10 stimuli with a 2-s interstimulus interval. Each condition was presented in a random order separated by a 60-s delay.

**Data Analysis.** Mean motor and sensory responses to step profiles were pooled for each stimulus duration and for each amplitude for all subjects. For triangular stimuli the initial ramp gradients of triangular waveforms were calculated from amplitude and frequency to reflect nerve loading rates (mm/s) equivalent to compression rate. Triangular stimulus frequencies were constant across all 12 subjects, but the stimulus amplitudes varied according to the depth of the groove between the medial epicondyle and olecranon of each subject. Therefore, absolute loading rates for triangular stimuli were not constant across all 12 subjects. However, for six subjects in whom the three amplitudes were 4, 6, and 8 mm, the motor and sensory responses to triangular stimuli were pooled so the effects of loading rate could be examined quantitatively.

Means and standard errors were calculated for each condition. Sensory ratings, CMAP amplitudes, and CMAP latencies for step stimuli were analyzed using two-way analysis of variance (ANOVA) for stimulus amplitude and duration, with Student–Newman–Keuls (SNK) post-hoc test for multiple comparisons. Data for the triangular stimulus (six subjects) were analyzed using two-way ANOVA for stimulus amplitude and loading rate, with SNK post-hoc test for multiple comparisons. Differences were considered significant when \( P < 0.05 \). Sensory responses and CMAP amplitudes to the maximum amplitude triangular stimuli were further analyzed using linear regression.

**RESULTS**

Adequate mechanical stimulation of the ulnar nerve was achieved in all 12 subjects, producing both motor and sensory responses, although not for all stimuli. In general, larger displacements were required to evoke motor responses (CMAPs) than sensory responses (paresthesias). Sites of perceived sensory activation were restricted to the ulnar innervation territory of the hand. Because responses in ADM were qualitatively similar to those in FDI, only the latter were analyzed quantitatively. Although at times stimuli were perceived as strong, no subjects reported pain. An example of raw data from one subject is presented in Figure 2. CMAP data are shown for FDI. In some traces, 50 Hz noise is present.

**Stimulus Amplitude.** Low- and medium-amplitude triangular stimuli did not always produce detectable CMAPs, but did produce paresthesias in many subjects. Conversely, the step stimuli generally produced pronounced CMAPs and a greater intensity of paresthesias. Mean sensory percepts and CMAP amplitudes for the three-step stimulus amplitudes are presented for the five-step stimulus durations in Figure 3A,C. There was a significant main effect of stimulus amplitude on sensory percepts \( (P < 0.001) \) and CMAP amplitude \( (P < 0.001) \). Sensory responses were significantly different between all three stimulus amplitudes \( (P < 0.05, \text{SNK post-hoc test for multiple comparisons}) \). CMAP amplitudes were significantly different between minimum- and medium-amplitude step stimuli, and between minimum- and maximum-amplitude step stimuli \( (P < 0.05, \text{SNK post-hoc test for multiple comparisons}) \).
The magnitude of the step stimulus did not significantly affect the onset or peak latency of the CMAP.

**Stimulus Duration.** The effect of stimulus duration was assessed using the step stimuli with widths of 0.5, 0.25, 0.167, 0.133, and 0.1 s. Mean amplitudes of the sensory and motor responses for the different durations are presented for the three-step stimulus amplitudes in Figure 3B,D. There was no main effect or interaction of stimulus duration on sensory or motor responses. This is exemplified in the representative raw data in Figure 2B.

We saw no “off” CMAP responses (i.e., responses to unloading of the nerve) during the retraction phase of any of the stimuli, nor any sustained activity during the static phase of the stimulus. There was no main effect or interaction of stimulus duration on CMAP onset or peak latencies. This result confirms that the evoked response was temporally coupled to the onset of the stimulus, i.e., to the dynamic phase of nerve compression but not to decompression.

**Loading Rate.** Mean sensory and motor responses to the maximum amplitude (8 mm compression) of the triangular stimulus for the six subjects with comparable loading rates are shown in Figure 4A,B. For these subjects, there was no main effect or interaction of loading rate on motor or sensory responses. However, there was a significant increase in mean intensity of paresthesias ($R^2 = 0.966, P < 0.005$) and CMAP amplitudes.
(R² = 0.934, P < 0.01) with increasing loading rate for the largest amplitude of triangular stimuli. There was no significant increase in CMAP amplitude or paresthesia rating with increasing loading rate for medium amplitude (6 mm) and low amplitude (4 mm) triangular stimuli.

There was a significant main effect of loading rate on the onset (P < 0.001) and peak (P < 0.001) CMAP latencies for triangular stimuli, CMAP onset, and peak latencies being inversely related to loading rate. However, the instantaneous stimulus amplitude at the CMAP onset did not vary with loading rate.

**DISCUSSION**

We used position-controlled dynamic compression of the ulnar nerve at the elbow to demonstrate that both cutaneous and motor axons exhibit mechanosensitivity to compressive loading but not unloading. Moreover, the mechanosensitivity of the sensory axons appeared to be greater than that of motor axons. There were no changes in CMAP onset or peak latencies with changes in stimulus amplitude or duration. There were significant decreases in CMAP onset and peak latencies with an increase in loading rate, but, regardless of the loading rate, the instantaneous stimulus amplitude at CMAP onset was con-
stant for each subject. Furthermore, there was a significant increase in sensory and motor amplitudes with loading rate, but only when assessed at the highest stimulus amplitude. Because of their higher loading rate, step stimuli were more effective in evoking responses than triangular stimuli of the same amplitude.

Methodological Considerations. The loading parameters were chosen to best accommodate anatomical variation between subjects and the capabilities of the experimental arrangement. There is some uncertainty about the size of the ulnar nerve within the groove (Fig. 5A). The measured depth did not necessarily correspond to the size of the subject and we could not be certain as to whether subjects with larger groove depths had proportionally larger ulnar nerves. Therefore, stimulus amplitudes were constant displacement rather than being determined as a percentage of the measured groove depth. The step stimulus durations were selected from preset options in order to cover a range of durations within the time of interest for acute compression (<1 s). Likewise for the triangular waveforms, this meant that the loading rate was larger for subjects with larger measured groove depths. For this reason, loading-rate results were analyzed only for six subjects that had the same measured groove depths and were therefore stimulated with the same loading rates. It is also possible that the position of the elbow (flexed to 90°) may have changed the thresholds to mechanical stimulation because of stretch (and presumably an increase in tension) of the ulnar nerve. Finally, we acknowledge that a more direct comparison of the evoked motor and sensory responses could be performed by comparing CMAPs with sensory nerve action potentials (SNAPs) obtained by recording the antidromic sensory discharges directly via a microelectrode in a cutaneous fascicle of the distal ulnar nerve. Preliminary

![FIGURE 4. Average paresthesia ratings and CMAP amplitudes for increasing loading rate for six subjects stimulated at the same maximum amplitude. (A) Mean ± SEM of paresthesia ratings (for six subjects with comparable loading rates) for five stimulus loading rates at the maximum amplitude (8 mm) of triangular stimuli (P < 0.005, R² = 0.966). (B) Mean ± SEM of CMAP amplitudes (for six subjects with comparable loading rates) for five stimulus loading rates at the maximum amplitude (8 mm) of triangular stimuli (P < 0.01, R² = 0.934).](image)

![FIGURE 5. Possible anatomical variation between subjects and possible mechanism for generation of mechanically evoked discharges. (A) It is uncertain whether subjects with larger groove depths have proportionally larger ulnar nerves or simply more overlying tissue. (B) We propose that dynamic axonal compression produces localized stretch of the lipid bilayer around the site of the imposed compression, indicated by the distortion of the grid. Since neural tissue is viscoelastic, a high strain rate causes increased resistance to deformation. The deformation would instead be taken up by ion channels, allowing ions to enter the cell, thereby causing depolarization.](image)
with maximal changes occurring at the medial epicondyle. Winklestein and DeLeo found separate thresholds for discrete and localized stretch either side of the site of compression, suggesting these can evoke paresthesias. However, despite the ulnar nerve normally being exposed to significant compression when the elbow is flexed \( \geq 90^\circ \), with maximal changes occurring at the medial epicondyle, ectopic action potentials generally do not occur. This compression is accompanied by a change in area of the nerve of up to 50%, involving a flattening of the nerve trunk and a significant increase in intraneural pressure at \( 90^\circ \) (the angle of elbow flexion used in the current study) but no focal compression and little change in extraneural pressure until \( 100^\circ \) flexion. Complete obliteration of function can occur in healthy human median nerves at modest external compressive loads \( \leq 50 \text{ mmHg} \). Watanabe et al. demonstrated that a stretch applied over a long period of time (2 N for 1 h) can be pathological when applied repeatedly over the same period for much shorter durations, i.e., with greater episodes of dynamic stretch.

Mechanisms. A number of possible mechanisms could explain the evoked responses to compression of a peripheral nerve. It can be speculated that mechanical deformation causes channels to open in the axonal membrane, allowing \( \text{Na}^+ \) ions to enter the cell, depolarize the membrane, and initiate action potentials (Fig. 5B). Alternatively, an unidentified mechanically gated channel may be responsible.

Based on our observations and an earlier suggestion, the compression stimulus may not be directly responsible for the evoked discharges. Rather, highly localized stretch either side of the site of compression may have a greater effect than the compression itself (Fig. 5B). Interestingly, Winklestein and DeLeo found separate thresholds for discrete and sustained responses from mechanical deformation of nerves. The biomechanical properties of neural tissue could explain some results of this study. Neural tissue is known to be viscoelastic, and its properties are highly dependent on the rate of deformation (strain rate). It has been demonstrated in numerous studies of neural tissue that greater stresses are developed along a nerve when it is loaded at higher strain rates (for a given strain). It has also been demonstrated in studies of mechanosensitive channels that an increase in pressure (or stress) used to elongate the membrane is associated with an increased probability of ion-channel opening (reviewed elsewhere). These findings are consistent with those of the current study, in that an increase in loading rate was accompanied by an increase in motor and sensory evoked responses. The increased loading rate would cause an increased stress in the nerve. This would cause an increased probability of ion channels opening, thereby increasing the likelihood of evoked responses. Furthermore, higher amplitudes of compression would likely deform a greater number of axons, thereby increasing the intensity of compound motor and sensory responses.

Finally, the viscoelastic behavior of neural tissue may explain the lack of “sustained” and “off” responses immediately following mechanical deformation. When viscoelastic tissues are deformed and held, the force required to maintain the deformation reduces with time. If the force is held constant, the elongation decreases with time. This has been demonstrated in various types of neural tissue. This relaxation behavior would reduce the probability of ion-channel opening, and depolarization would cease immediately after deformation.

Although we propose that the lipid bilayer of the axonal membrane is responsible for the transfer of deformation, it is possible that the cytoskeleton plays a role. The cytoskeleton is important for mechanotransduction in many cell types, for example, endothelial cells (see Alenghat and Ingber for a review of the role of the cytoskeleton in mechanotransduction), but its role in mechanotransduction in axons has not been investigated. Furthermore, although a recent study of a bacterial mechanosensitive channel, MscS, suggests that the channel itself may be sensitive to changes in loading rate, it is not clear that the lipid bilayer effects can be separated from the channel’s response.

Differences between Sensory and Motor Axons. We observed that sensory responses were evoked at lower loading rates and amplitudes than those producing a motor response. This may be related to the known biophysical differences between sensory and
motor axons which, when considered together, suggest that sensory axons are more readily excitable at rest than motor fibers. The factors underlying this include more persistent sodium channels and greater inward rectification and the lower rheobase. These axons therefore have a longer strength–duration time constant, which means that a sensory axon of the same diameter as a motor axon will be more excitable.

Sensory axons, moreover, are more likely to demonstrate ectopic activity and are less susceptible to conduction block and more susceptible to ischemia than motor axons. We do not believe our results are secondary to local ischemic changes during compression. First, changes in axonal excitability due to ischemia are not seen until after ~30 s of nerve compression and were normally accompanied by sensory ectopic activity. Second, all subjects reported discrete sensory events without the perception of sustained pressure. Third, the immediate response of motor fibers gave a single CMAP at the stimulus onset without apparent repeated firing of the same axons, in addition to an absence of both prolonged CMAPs or an off-response.

The mechanism proposed in this study may also help to explain the symptoms associated with common syndromes or injuries. One such example is Tinel’s sign, in which paresthesias in the hand are commonly reported by those with carpal tunnel syndrome when the median nerve is tightly tapped at the wrist. In this instance chronic compression of the nerve may produce a prior, sustained deformation preload, imparting a higher resistance to any subsequent deformation and lowering the threshold of these axons to mechanical stimulation.

The lower mechanical threshold of sensory axons seen in this study also implies that a small extent of dynamic nerve compression may cause sensations such as paresthesias, without any physical symptoms such as muscle fasciculation or cramping. This may be important for diagnosis of conditions in which low-intensity, repeated, dynamic nerve deformation produces abnormal sensations without motor symptoms.

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Dynamic Nerve Compression
ABSTRACT: The patterns of normal daily activity that are required to maintain normal skeletal muscle properties remain unknown. The present study was designed to determine whether spinal cord isolation can be used as a reliable experimental model of neuromuscular inactivity, that is, as a baseline for the absence of activity. Electromyograms (EMGs) were recorded from selected hindlimb muscles of unanesthetized rats over 24-hour periods before and 7, 30, 60, and 90 days after surgical isolation of the lumbar spinal cord. Our data indicate that some rat slow muscle fibers pre-surgery were activated for less than 3 hours per day. Spinal cord isolation (SI) reduced the mean daily integrated EMG (IEMG) and daily EMG duration in the primary slow extensor muscle (soleus) to $<1\%$ of control, and in the primary fast extensor muscles [medial gastrocnemius (MG) and vastus lateralis (VL)] to $<2\%$ of control. These parameters were decreased to $<8\%$ and $3\%$ of control, respectively, in a primary fast flexor muscle, the tibialis anterior (TA). From 30 to 90 days post-SI, the mean amplitudes of the spontaneous EMG bursts were relatively normal in the soleus, increased $\sim 2\text{-fold}$ in the MG and VL, and increased $\sim 4\text{-fold}$ in the TA. Some evidence of the normal antagonistic flexor–extensor relationship was apparent in the brief periods of recorded activity post-SI. These results indicate that SI eliminates nearly all of the normal EMG activity in the hindlimb muscles in the presence of relatively normal muscle innervation and functional intraspinal neural circuitry.


IS SPINAL CORD ISOLATION A GOOD MODEL OF MUSCLE DISUSE?

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One approach to understanding the role of neuromuscular activity, that is, activation and loading, in maintaining skeletal muscle properties has been in having an experimental model from which a known baseline for the levels of activity can be reliably assumed. This has been attempted by a number of experimental manipulations such as denervation\textsuperscript{1,16,29,45}, chronic nerve blockade with tetrodotoxin (TTX)\textsuperscript{4,22,40}, or spinal cord isolation (SI), that is, isolating a portion of the spinal cord via complete spinal cord transections at two levels plus bilateral dorsal rhizotomy between the transection sites.\textsuperscript{5,17,42} In all of these studies there has been little or no verification of the level of success in eliminating neuromuscular activity. We have been using the SI model in the rat because: (1) the connectivity between the muscle and the innervating motoneurons is intact, thus maintaining neural activity–independent neurotrophic factors; (2) the motoneurons, as well as the muscles, appear to be inactive; and (3) the animals can be maintained in a healthy state for prolonged periods.\textsuperscript{23,33,37}

Previous work suggests that SI in the adult cat results in almost complete electrical silence in the affected muscles. For example, Steinbach et al.\textsuperscript{39} observed low levels of electromyographic (EMG) activity during acute recordings (between 10 min and
Rat Hindlimb Muscle Inactivity

4 hours on three occasions with the cats awake and unrestrained) from the flexor hallucis longus and the soleus muscle at about 500 days and from the tibialis anterior and lateral gastrocnemius at about 700–900 days after SI in cats. Similar results were observed in the tibialis anterior and flexor hallucis longus at about 210 days after surgery. In addition, no EMG activity could be evoked by tactile stimulation nor was there any EMG activity recorded when the cats propelled themselves with their forelimbs. Only one previous study has quantified EMG activity from SI animals. Chronic EMG electrodes were implanted in the extensor digitorum longus muscle of four SI cats during the last week of a 6-month experimental period. The mean total integrated EMG activity recorded during two 24-hour sessions from each SI cat was 0.003% of normal control values. However, similar data are not available for the rat model.

The primary purpose of the present study was to determine whether the SI procedures result in electrically silent hindlimb muscles in rats. EMG activity was recorded for 24-hour periods from a predominantly slow (soleus) and fast (medial gastrocnemius, MG) ankle plantarflexor, a predominantly fast ankle dorsiflexor (tibialis anterior, TA), and a predominantly fast knee extensor (vastus lateralis, VL) before and at several time-points up to 90 days after SI. The results clearly indicate that SI is a model of near, although not absolute, skeletal muscle inactivity in rats.

METHODS

Animals and Experimental Groups. Seven adult female Sprague–Dawley rats (mean body weight: 238 ± 8 g) were used for this study. The studies were performed in two series: the initial series involved EMG recordings from three SI rats pre- and 7, 30, 60, and 90 days post-SI, and the second series involved EMG recordings from four SI rats pre- and 7 and 30 days post-surgery. Initial statistical comparisons showed no differences between the two series of rats and thus the data were combined for all subsequent analyses. All procedures were approved by the UCLA Chancellor’s Animal Research Committee and followed the animal care guidelines of the American Physiological Society.

EMG Implant Procedures. EMG implants were done approximately 2 weeks prior to SI surgery. A skin incision was made along the sagittal suture of the skull. The scalp musculature and underlying connective tissue was reflected laterally and the exposed skull was dried thoroughly. Three screws were anchored firmly to the skull and a 9-pin (gold-plated) amphenol connector was cemented (dental cement) to the skull and screws. Eight multistrand Teflon-insulated stainless-steel wires (15 strands, 50-μm gauge; Cooner Electronics) were led subcutaneously from the connector to the hindlimb (see below). About 1 cm of the Teflon coating was removed from the distal end of the ninth wire, which was then embedded in the middle-back region and served as a common ground. The undersurface of the headplug between the pins and the wires had been sealed with epoxy to prevent any body fluid seeping into the contact area.

Skin incisions were made in the hindlimb to expose the soleus, MG, TA, and VL muscles. Two wires from the headplug were inserted into each of the following: the midbelly of the soleus and a deep region (i.e., close to the bone) of the midbelly of the MG, TA, and VL. These anatomical locations were chosen to assure a consistent sampling site for all rats and to sample a predominantly slow fiber type area in the soleus, and a mixed area (~30%–35% slow fibers) in the deep regions of the MG, TA, and VL. The wires were inserted into each muscle region (~2–3 mm apart) by passing them individually through a 23-gauge hypodermic needle. Recording electrodes were made by removing ~0.5–1.0 mm of insulation from each wire. Following back-stimulation of the muscle through the headplug to ensure proper placement of the electrodes, each lead was secured with a suture at its entry and exit from the muscle. This procedure effectively secured the electrodes in the muscle belly. The bared tips of the wires were covered by gently pulling the Teflon coating over the tips to avoid recording extraneous potentials. All incisions were closed using 4-0 Ethilon suture. These procedures have been used routinely in our laboratories.

SI Surgical Procedures. The SI surgical procedures are a modification of the original protocols by Tower and these procedures and the care for the SI rats have been detailed previously. Graphical depictions of the SI procedures have also been published. The rats were anesthetized with ketamine hydrochloride (100 mg/kg body weight) and xylazine (5 mg/kg body weight) administered intraperitoneally. Supplemental doses (30% of the initial dose of ketamine hydrochloride intraperitoneally) were given as needed. Under aseptic conditions, a longitudinal midline skin incision was made dorsal to the spinal column from the T6 to the L6 vertebral levels and a partial laminectomy was performed be-
between vertebral levels T7 and L5. After opening the dura, the dorsal roots were cut intradurally bilaterally from the mid-thoracic to S1 spinal cord level. A long portion of each dorsal root—that is, from the surface of the spinal cord to as close to the exit of the root from the vertebral column as possible—was removed to assure a complete rhizotomy. Lidocaine hydrochloride (1%; one or two drops) was applied to the transection sites. The spinal cord was lifted gently with a curved probe or fine forceps and completely transected at both mid-thoracic and high sacral spinal cord levels using micro-dissection scissors. Gel foam was packed between the cut ends of the spinal cord at each transection site. A strip of gel film then was placed along the length of the exposed spinal cord. The paravertebral muscles and fascia surrounding the spinal column were sutured using 4-0 Ethilon. The rhizotomy margins were trimmed carefully with a curved probe or fine forceps and completely transected. A strip of gel film then was placed along the length of the exposed spinal cord. The paravertebral muscles and fascia surrounding the spinal column were sutured using 4-0 chromic gut and the skin incision was sutured using 4-0 Ethilon.

The rats were allowed to recover fully from anesthesia in an incubator (27°C) and were given lactated Ringer solution (5 ml, subcutaneously). Polyclinic, a general antibiotic, was administered (100 mg/kg, subcutaneously, twice per day) during the first 3 days of recovery. The rats were housed individually in polycarbonate cages (10.25 in. × 18.75 in. × 8 in.) and the room was maintained at 26 ± 1°C, 40% humidity, and on a reversed 12:12 hour light: dark cycle. Post-surgical care involved manual expression of the bladder three times per day for the first 7 days and twice per day thereafter. On a daily basis, cage bedding was changed to prevent skin infections, animals were assessed for health (e.g., body weight, urination, defecation, and hydration), the hindlimbs were manipulated passively through a full range of movement to maintain joint flexibility, and reflex testing on the hindlimbs was performed (i.e., withdrawal reflex and toe-spread response). Rats were supplied Purina rat chow and water ad libitum and were given pieces of fresh fruit daily. Following the last recording session (see below), the completeness of the spinal cord transections and of the dorsal rhizotomies was verified visually. All transections and rhizotomies were complete for the rats used in this study.

**EMG Recording and Analysis.** All EMG recordings were performed using the same cages in which the animals were normally housed. A nine-conductor swivel (Alice King Chatham Medical Arts, Inglewood, California) was mounted on the top of the cage, allowing the animals to move freely during the recordings. Signals were amplified (×1000, custom-built portable amplifiers) and then recorded digitally at 2 kHz using customized acquisition software. Recordings began between 8:00 a.m. and 11:00 a.m. and concluded 24 hours later. All normal animal care activities were maintained during the recording period (i.e., expression of the bladder and daily health checks) so that any increase in activity during these periods would be included. Animal care activities during the dark period were performed under “red light” to minimize any disturbance of the rats. Recordings were performed between 4 and 7 days prior to SI surgery, and 7, 30, 60, and 90 days post-SI.

The EMG data were analyzed using in-house software developed using LabView (National Instruments, Houston, Texas). The methods have been reported elsewhere. Briefly, all raw EMG data were first reviewed at time resolutions ranging from fractions of a second to several minutes of data to identify and exclude segments of data containing interference (noise). The remaining data were digitally high-pass filtered at 10 Hz and rectified. Mean EMG values of 40-ms time epochs were calculated from these data, effectively smoothing with a 12.5-Hz low-pass filter and decimating the data to 25 samples per second. Amplitude histograms were constructed from the processed EMGs signals from each muscle for each hour of the day. Integrated EMG (IEMG) values were calculated by multiplying each bin count by its corresponding amplitude and summing the result over all bins above a threshold level. The duration of EMG activity was calculated by summing all bin counts above a threshold level. The threshold level was determined by generating amplitude histograms of EMG data when no activity was apparent in any muscle and the animal was assumed to be inactive. The threshold level was set at the highest bin required to exclude 95% of the baseline data. Mean amplitudes of EMG activity were calculated by dividing the integral by the duration. Data from the pre-SI recordings were adjusted to a 24-hour period in those instances where interference excluded some data from our analysis. The post-SI data were treated slightly differently because the recordings indicated that the muscles were inactive for the majority of the time. In this case, considerable time was saved in determining the IEMG by analyzing only recordings containing EMG activity and adding the remaining time in each hour to the zero-amplitude bins of the amplitude distributions. The threshold level was determined as described above, and the duration and mean EMG were calculated in the same manner as described above for the IEMG.

**Video Recordings.** At selected time-points after SI surgery, we video-recorded the rats for periods of...
5–6 hours during cage activity (using red light during the dark period), manual bladder expressions, and reflex testing. The EMG recordings were synchronized with the video using a time-code generator.

**Statistical Procedures.** The data are reported as mean ± SEM. For the pre-SI data, a mixed analysis of variance model was used to compare each EMG parameter among the four muscles. For the post-SI data, a time scatterplot with a Loess regression curve was produced and a first-degree spline model was developed for each EMG parameter. The slopes were not significantly different between days 30 to 60 and days 60 to 90, and thus these were combined into a single slope. Regression lines between days 7 to 30 and days 30 to 90 then were compared. The level of significance was set at $P < 0.05$.

**RESULTS**

**General Observations.** As expected and reported previously, the SI rats lost body weight during the initial week after SI surgery and then gained body weight during the remainder of the study. Throughout the study there was no response to reflex testing, rubbing the stomach, or toe or tail pinching, and the hindlimbs were completely flaccid in all SI rats. Based on the video recordings, the rats were quite active in their cages. The rats used their forelimbs to move around the cage to access their food and water ad libitum. Some instances of EMG activity were observed in the hindlimb muscles when the rats were moving across the cage using their forelimbs, even though there was no observable movement in the hindlimbs. There often was some EMG activity recorded during the bladder expressions in SI rats, both during the handling of the rat and during the bladder manipulation itself. Some bursts of spontaneous activity also were observed when the rats were quiescent or appeared to be sleeping.

**Overall EMG Characteristics Pre- and Post-SI.** Figure 1 illustrates representative EMG signals recorded during normal cage activity pre-surgery (Fig. 1A) and post-SI (Fig. 1B and C). The soleus muscle showed high activity levels pre-surgery even when the other muscles were relatively quiet, reflecting its normally high excitability level (Fig. 1A). The fast extensors (MG and VL) showed less activity than the soleus, and the fast flexor (TA) showed the least amount of activity for any muscle studied. There were numerous periods of alternating activity between the extensor (soleus, MG, and VL) and flexor (TA) muscles, most likely while the rat was walking around in the cage (see left side of the traces in Fig. 1A) and periods when the soleus was the only muscle highly active, most likely when the rat was standing quadrupedally (see right side of traces in Fig. 1A). Following the SI surgery, the incidence of EMG activity in these hindlimb muscles was found to be minimal. Three types of activity were observed in the muscles post-SI: (1) simultaneous short, high-amplitude bursts of activity in all muscles (Fig. 1B); (2) single episodes of extensor–flexor alternating activity (Fig. 1C); and (3) sporadic bursts in individual muscles.

**Daily EMG Activity Levels in Rats Pre-SI.** Pre-surgery data are presented for seven rats. It should be noted that data from three of these rats are the same as those reported by Hodgson et al. These data, however, are included herein because they provide the baseline data for calculating the percent activity post-surgery for each of these three rats. For all EMG measures, the soleus was the most active and the TA was the least active of the muscles monitored (Fig. 2). The daily IEMG was 2.7-, 11.3- and 3.5-fold higher in the soleus than in the MG, TA, and VL, respectively (Fig. 2A). The mean daily duration of activity
was 1.6-, 4.6-, and 2.1-fold higher in the soleus than in the MG, TA, and VL, respectively (Fig. 2B). The mean EMG burst amplitude in the soleus was 1.8-, 2.3-, and 1.6-fold higher for the same muscles (Fig. 2C). The activity for all muscles showed a diurnal cycle with higher integrals (63%–73%) and durations (63%–70%) during the dark than light period (Fig. 2A and B, respectively).

**Daily EMG Activity in Rats Post-SI.** SI surgery resulted in flaccid paralysis of the hindlimbs and this was reflected in the very low levels of EMG activity observed after, compared to before, surgery (Fig. 3). There was no evidence for a diurnal cycle in any muscle post-SI and the overall response after SI was somewhat different across the muscles studied.

**Soleus: Slow Extensor.** Both the mean total daily IEMG (Fig. 3A) and the mean total EMG duration (Fig. 3B) of the soleus were <1% of the pre-SI levels at all time-points post-SI. These two parameters were the lowest at 7 days post-SI, specifically 0.04% and 0.16%, respectively. Mean EMG burst amplitude (Fig. 3C) was ~25% of pre-SI values at 7 days post-SI and then progressively increased to reach ~110% at 90 days post-SI.

**MG and VL: Fast Extensors.** The total daily IEMG (Fig. 3D and J) and the total EMG duration (Fig. 3E and K) were less than ~2% and 1%, respectively, at all time-points post-SI in the MG and VL. These values were lowest at 7 days post-SI; that is, 0.21% and 0.10% and 0.09% and 0.09% for the MG and VL, respectively. Mean burst amplitude (Fig. 3F and L) was near pre-SI values at 7 days, and ~2–3-fold higher thereafter.

**TA: Fast Flexor.** In general, the TA post-SI EMG measures relative to pre-surgery were higher than each of the other muscles studied. Total daily IEMG values (Fig. 3G) were <1% at 7 days, but increased to ~8%, 6%, and 4% at 30, 60, and 90 days post-SI. Total daily EMG duration (Fig. 3H) was ~2% or less at all time-points, with the lowest value at 7 days, that is, 0.4%. The mean burst amplitude (Fig. 3I) was ~2-fold higher than the pre-SI value at 7 days and between 3.5- and 4-fold higher thereafter.

The ranges in absolute values for the EMG parameters pre-SI and 7 and 30 days post-SI are shown in Table 1. These two post-SI time-points were chosen because, in general, the lowest and highest EMG values post-SI were observed at 7 and 30 days, respectively. The ranges highlight the animal-to-animal variability for all EMG parameters. They also demonstrate that all muscles have very similar amounts of residual activity post-SI; that is, the ranges in daily IEMG and daily EMG duration across muscles are much more similar after than before SI.

**DISCUSSION**

The primary purpose of this study was to determine whether the SI model can be used as a reliable and valid model of skeletal muscle inactivity. The SI procedure reduced the overall muscle activity from several hours per day to a few isolated bursts, most likely under no load or low-load conditions, occupying a few minutes per day. These data suggest that SI can be used as a model for identifying influences by the motoneuron on skeletal muscles that are mediated neurally through activity-independent mechanisms. This model differs in important ways from other presumed models of skeletal muscle inactivity, such as paralysis induced via TTX application to the peripheral nerve and denervation.14,41 For example, following TTX treatment the motoneurons theoretically would have near-normal activation levels that...
could influence the neurotrophic influences on the muscles. In the SI model, the low level of motoneuron activity is reflected in the EMG recordings of the present study. Denervation theoretically reduces the neuromuscular activity levels to nearly zero, but this model also eliminates all neurotrophic influences.

**Table 1.** Ranges for daily IEMG, daily EMG duration, and mean burst amplitude for each muscle pre- and post-SI.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Daily IEMG (mV.s)</th>
<th>Mean burst amplitude (µV)</th>
<th>Daily EMG duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre 7 days 30 days</td>
<td>Pre 7 days 30 days</td>
<td>Pre 7 days 30 days</td>
</tr>
<tr>
<td>Sol</td>
<td>787–3933 0.04–1.98 3.0–15.4</td>
<td>22.1–87.8 5.5–16.2 10.2–31.6</td>
<td>593–874 0.08–3.22 2.34–12.50</td>
</tr>
<tr>
<td>MG</td>
<td>289–1401 0.01–3.93 3.8–15.5</td>
<td>13.7–34.1 5.5–66.2 25.8–64.7</td>
<td>103–684 0.02–0.99 1.73–5.25</td>
</tr>
<tr>
<td>TA</td>
<td>94–198 0.05–1.81 8.4–24.8</td>
<td>12.3–22.3 11.5–64.1 54.6–91.8</td>
<td>70–192 0.07–1.16 1.60–5.89</td>
</tr>
<tr>
<td>VL</td>
<td>185–944 0.01–1.25 2.5–8.6</td>
<td>15.7–37.7 5.4–38.9 22.7–75.1</td>
<td>156–722 0.02–0.55 0.91–4.88</td>
</tr>
</tbody>
</table>

IEMG, integrated electromyography; SI, spinal cord isolation; Sol, soleus; MG, medial gastrocnemius; TA, tibialis anterior; VL, vastus lateralis.
**Daily Muscle Activity Levels Pre-SI.** The general activation patterns of the hindlimb muscles during cage activity were similar to those detailed recently by Hodgson et al. All muscles showed a diurnal cycle of activity with a majority of the activity occurring during the dark period of the cycle, reflecting the nocturnal nature of this species. Based on the daily IEMG and EMG duration levels, the primarily slow extensor (soleus) had the highest, the primarily fast extensors (MG and VL) had a moderate, and the primarily fast flexor (TA) had the lowest activity levels. These observations are consistent with the soleus having a strong antigravity function and being active while the rat is standing or walking around the cage. The moderate amount of activity in the MG and VL is consistent with the secondary role of fast extensor muscles during postural and low-level activity. The relatively small amount of activity in the TA reflects its primary role as an ankle flexor that is recruited mainly during the swing phase of the step cycle during quadrupedal locomotion. The mean EMG burst amplitude of the soleus also was the highest of all muscles studied, reflecting the relatively high recruitment level of this muscle during routine cage activity. These observed relative activation patterns of the soleus, MG, and TA during cage activity are consistent with those recorded during treadmill locomotion at various speeds and inclines. In addition, the relative daily activity levels generally are consistent with a number of previous chronic EMG studies in rats.

**Daily Muscle Activity Levels Post-SI.** The most striking effect of SI was the reduction of the total daily IEMG and EMG duration to <1% of control levels for all muscles on day 7 post-SI. This amounted to less than 1 minute during which some activity above baseline could be detected throughout an entire 24-hour period. There was a very slight recovery of total daily activity duration and IEMG to ~2% of pre-SI values in the extensor muscles during the 3-month period over which recordings were made. TA activity recovered to a higher level (a maximum of 8% at 30 days post-SI), with a large part of this recovery due to a 4-fold increase in burst amplitude relative to pre-SI values. The overall result of SI appears to be an almost complete abolition of EMG activity in the selected extensor and flexor muscles of the hindlimb, particularly in terms of duration of activity. This inactivity is produced under conditions in which the muscle–motoneuron connectivity remains intact and where the innervating neurons have not been subjected to pharmacological manipulation. Therefore, the SI preparation provides a known, very low baseline activity level for experiments designed to investigate neural activity–dependent vs. –independent mechanisms that modulate the properties of skeletal muscle fibers.

The short bursts of activity that remained after SI generally lasted for a few milliseconds, with long intervals of inactivity between bursts. Many periods of activity coincided with the handling of the animals; for example, hindlimb movements were observed when the rats were manipulated to void their bladder. Attempts to elicit reflexes by pinching or pulling the foot during these periods, however, resulted in no detectable responses in the legs. It appears that these brief periods of activity were the result of mechanical perturbation of the isolated spinal cord.

There is an important point to note in the significance of the higher values of total daily IEMG and daily duration of activity for the TA compared to the other muscles. The absolute mean daily IEMG at each post-SI time-point was quite similar for the four muscles studied (Table 1). Since the TA was the least active muscle pre-SI, this resulted in a relatively high percentage of activity post-SI compared to pre-SI. However, the absolute duration of TA activity was markedly reduced post-SI and was similar to that of the other muscles post-SI. It should be noted that the effects of SI on fiber size and phenotype were less in the TA than the MG, perhaps reflecting the relatively lower activity level pre-SI and the somewhat higher relative residual activity in the TA than MG post-SI.

**Mean EMG Amplitudes Post-SI.** The mean amplitude of the EMG bursts was smaller post- than pre-SI only in the soleus muscle after 7 days of SI (35% of pre-SI values). Soleus EMG amplitudes returned to pre-SI levels within 30 days of the SI surgery and remained at these levels thereafter. At 7 days post-SI, the MG and VL EMG amplitudes were similar to the pre-SI values and the mean amplitudes were approximately doubled in subsequent recording sessions. The TA mean EMG amplitude was doubled at 7 days post-SI and increased to 3–4 times pre-SI values in the later recordings. Therefore, the mean EMG burst amplitude remained relatively normal, and in most cases was even higher than normal after SI. The apparent reason for the higher mean EMG amplitude after than before the SI surgery is that almost all of the low-level activity is eliminated post-SI. Thus, only bursts of activity, with relatively high amplitudes, were present after SI, resulting in higher mean EMG amplitudes. Another physiological indicator of the functionality of the spinal circuitry after SI surgery was the synergistic and antagonistic relationships be-
between the extensor and flexor muscles (Fig. 1C), suggesting that the spinal circuitry generally associated with central pattern generation remained relatively normal after SI.

Possible Explanations for Residual Activity in Muscles of SI Rats. As described in the Methods section, the SI surgery eliminates all descending input from the higher centers, all peripheral sensory input entering through the dorsal roots, and all ascending input through the spinal cord below S1–S2 to the isolated region of the spinal cord. Thus, what is the source of the small amount of residual activity in the muscles associated with the isolated region of the spinal cord? There are at least five possibilities.

A first possibility is that there is a direct mechanical activation of the neurons in the isolated region of the spinal cord. Although we were careful in placing a layer of gel film along the exposed dorsal surface of the spinal cord, we observed connective tissue adhesions from the newly formed tissues (connective tissue, cartilage, bone) at the sites of the midline partial laminectomy performed to identify the dorsal roots. It is highly likely that when the SI rats are handled during the daily bladder expressions, or when the rats twist their paralyzed hindquarters vigorously when using their forelimbs to move around in their cages, the movements result in connective-tissue adhesions “pulling” on the spinal cord and mechanically activating some of the neurons. This possibility is reinforced by our video recordings and observations showing a relatively high incidence of EMG activity during manual expression of the bladder or when performing motor activities in the cage environment.

A second possibility is that some activation of the motoneurons could occur from sensory input emanating from ventral root afferents. Although we have no direct evidence for this possibility, the presence of a number of ventral root afferents has been reported previously in rats, and ~10% of these are thought to be sensory fibers. However, it should be noted that electrophysiological studies have shown five possible courses for these afferent ventral root fibers: (1) actually entering the spinal cord via the ventral roots; (2) innervating the ventral spinal pia mater and/or the ventral root itself; (3) entering the ventral root and then looping back to enter the spinal cord through the dorsal root; (4) having a blind ending in the ventral root; and (5) entering the ventral root and then tapering out. Therefore, it appears that only a very small number of these ventral root afferents could impact the activation level of the motoneurons.

A third possibility is that the membrane properties of the motoneurons and interneurons in the isolated region of the spinal cord in rats could change and become highly excitable after the SI surgery. Our preliminary electrophysiological observations of motoneurons suggest that the active properties of the isolated motoneurons are unaffected, whereas their passive properties are somewhat more excitable after SI. We also have preliminary data showing that the size and the levels of succinate dehydrogenase activity of both alpha- and gammamotoneurons are unaffected after 30 days of SI in rats (Roy, Ishihara, Matsumoto, Zhong, and Edgerton, unpublished observations). Combined, these data are consistent with the view that motoneurons are highly resistant to changes in activity level.

There is evidence, however, that after a complete spinal cord transection the motoneurons spontaneously develop persistent sodium and calcium currents that are associated with the development of spasticity in chronic spinal animals. It is possible that such persistent internal currents are present in the motoneurons of SI rats, and that some of the spontaneous EMG data observed are due to the low threshold for excitation resulting from these adaptations.

A fourth possibility is that the net excitability of the interneurons increases post-SI. Most of the excitatory and inhibitory input to motoneurons is mediated from supraspinal and peripheral input via interneurons. Thus, these interneuronal pools eventually define the level of excitability of the motoneurons. These interneurons are known to have the capacity to excite motoneurons in a rhythmic and coordinated manner as a result of central pattern generation. The present data suggest that this capability increases post-SI, in that the mean EMG amplitudes are normal or greater than normal post-SI. In addition, the presence of alternating, coordinated flexor–extensor EMG bursts is consistent with interneuron activation.

A fifth possibility is an increase in the excitability of the muscle fibers themselves. Although there is no direct evidence for this occurrence in the SI model, there are some data from models of decreased use. The resting membrane potential of fibers from the extensor digitorum longus muscle of rats was decreased (~15 mV) between 7 and 30 days after a complete spinal cord transection at T6. In addition, cell membrane input resistance was decreased between 10 and 15 days post-transection. Hindlimb unloading results in a progressive increase in sodium current density in the soleus muscle over a 3-week period.

Stretch-sensitive ion channels have been...
identified in skeletal muscle membranes. Passive static stretch has been shown to increase calcium concentrations in skeletal muscle fibers in an in vitro preparation. All of these adaptations in the muscle fibers themselves associated with a reduction in neuromuscular activity levels could result in spontaneous depolarizations and account for some of the activity recorded post-SI.

**Perspective.** The SI model results in near inactivity of the hindlimb muscles associated with the motoneurons in the isolated region of the spinal cord. This level of inactivity is produced while maintaining intact neuromuscular connectivity. SI eliminated more than 98% of the hindlimb extensor total daily IEMG activity and more than 92% of the TA activity recorded post-SI.

This study was funded by National Institutes of Health, grant NS16333. The authors thank Maynor Herrera for excellent care of the animals, Manuel Campa for his assistance in data analysis, and He-jing Wang for her statistical expertise.

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ABSTRACT: Reducing-body myopathy (RBM) is a rare myopathy characterized by the presence of unique sarcoplasmic inclusions called reducing bodies (RBs). We characterized the aggresomal features of RBs that contained γ-tubulin, ubiquitin, and endoplasmic reticulum (ER) chaperones, together with a set of membrane proteins, in a family with hereditary RBM. Increased messenger ribonucleic acid and protein levels of a molecular chaperone, glucose-related protein 78, were also observed. These results suggest that the unfolded protein response caused by the accumulation of misfolded proteins in the endoplasmic reticulum plays an important role in the formation of RBs.


UNFOLDED PROTEIN RESPONSE AND AGGRESOME FORMATION IN HEREDITARY REDUCING-BODY MYOPATHY

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The endoplasmic reticulum (ER) is the site where newly synthesized secretory and membrane proteins are folded and assembled under a stringent quality-control system that prevents the development of aberrant conformers. The accumulation of misfolded/unfolded proteins in the ER leads to the unfolded protein response (UPR) that enhances folding capacity by transcriptional induction of ER chaperones and translationally represses protein synthesis. Misfolded proteins are removed from the ER by retrotranslocation to the cytosol and degradation by the ubiquitin–proteasome system. If these misfolded proteins fail to fold correctly and are not degraded by the proteasome, they are transported in a microtubule-dependent manner to the perinuclear microtubule-organizing center together with ubiquitin, ER chaperones, and form cytoplasmic aggregates called aggresomes. Aggresomes are usually surrounded by a cage of reorganized intermediate filaments and undergo autophagolysosomal degradation. Postmitotic cells, such as neurons and myocytes, are particularly vulnerable to the detrimental effects of aggresome accumulation because they cannot reduce potentially toxic substances through cell division.

Reducing-body myopathy (RBM) is a rare myopathy characterized pathologically by the presence of intracytoplasmic inclusion bodies strongly stained by menadione-linked α-glycerophosphate dehydrogenase (MAG) in the absence of substrate, α-glycerophosphate. The term “reducing body (RB)” implies the reducing activity of the inclusions to nitroblue tetrazolium in the absence of substrate. This condition is also commonly associated with rimmed vacuoles and cytoplasmic bodies. The clinical features of RBM are variable and can be classified into three forms, namely (1) severe infantile form, (2) benign congenital form, and (3) late onset form.

Abbreviations: endoplasmic reticulum stress-associated degradation; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; GRP, glucose-regulated protein; ER, endoplasmic reticulum; hRBM, hereditary reducing body myopathy; IBMFFD, inclusion body myopathy associated with Paget’s disease of bone and frontotemporal dementia; MAG, menadione-linked α-glycerophosphate dehydrogenase; mRNA, messenger ribonucleic acid; RB, reducing-body; RBM, reducing-body myopathy; RT-PCR, reverse transcriptase–polymerase chain reaction; sRBM, sporadic reducing-body myopathy; UPR, unfolded protein response; VCP, valosin-containing protein

Key words: aggresome; endoplasmic reticulum stress; endoplasmic reticulum stress-associated degradation (EDRA); reducing-body myopathy; unfolded protein response

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Most of the patients have sporadic disease and only a few familial cases have been reported. Here we report the aggresomal features of RBs found in a new family with hereditary RBM (hRBM).

MATERIALS AND METHODS

Patients. Details of the clinical features of this family with hRBM have been described elsewhere. Briefly, patient 1 is an 11-year-old boy of Japanese and Filipino descent in good health until 10 years of age, when he developed proximal-dominant muscle weakness and spinal rigidity. Serum creatine kinase was elevated to 495 IU/L (normal 70 IU/L), and a muscle biopsy was performed from the left biceps brachii at 11 years of age. Patient 2 is the mother of patient 1, a 35-year-old Filipino. She noticed asymmetrical generalized muscle weakness at 29 years of age and became wheelchair-bound 5 years later. No spinal rigidity was observed. Serum creatine kinase level was elevated to 417 IU/L, and muscle biopsy was performed at the age of 31 years.

Muscle specimens from both patients displayed scattered MAG-positive cytoplasmic inclusions in the absence of substrate, α-glycerophosphate. Some muscle fibers contained rimmed vacuoles. Atrophic fibers that partly clustered in groups and scattered cytoplasmic bodies were also seen in the specimen from patient 1. On electron microscopy, RBs frequently engulfed myonuclei and consisted of clusters of granular materials with electron density similar to chromatin.

Immunohistochemical and Western Blot Analyses. The antibodies used in this study are listed in the table which appears as supplementary material at http://www.mrw.interscience.wiley.com/suppmat/0148-639X/suppmat/.

Table 1. Results of immunoreaction of reducing body (RB) in the hereditary RB myopathy (hRBM) muscle.

<table>
<thead>
<tr>
<th>Protein category</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoplasm</td>
<td>None</td>
<td>Nuclei, nucleoli</td>
</tr>
<tr>
<td>Nuclear envelope</td>
<td>Emerin, Lamin A, Lamin C, LAP2</td>
<td>Lamin B</td>
</tr>
<tr>
<td>Centrosome</td>
<td>γ-tubulin (C)</td>
<td>None</td>
</tr>
<tr>
<td>UPR</td>
<td>IRE1α, p-PERK, GRP78, GRP94, Calnexin, ERp72, PDI</td>
<td>None</td>
</tr>
<tr>
<td>ERAD</td>
<td>VCP, Polyubiquitin, 26S proteasome P27 subunit (P)</td>
<td>None</td>
</tr>
<tr>
<td>Cytoplasmic chaperones</td>
<td>HS70</td>
<td>αB crystallin</td>
</tr>
<tr>
<td>Internal membranes</td>
<td>GM130, Limp1, LAMP2, SERCA1, SERCA2</td>
<td>None</td>
</tr>
<tr>
<td>Intermediate filaments</td>
<td>Desmin (P)</td>
<td>α-actinin, MHC fast, MHC slow, Titin, Telethonin</td>
</tr>
<tr>
<td>Sarcomere</td>
<td>Actin (P)</td>
<td>None</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>Dystrophin, α-, β-DG, α-SG, Dysferlin, Caveolin-3, nNOS, Integrin α7B, ILK, Paxillin,</td>
<td>Merosin, Collagen VI</td>
</tr>
<tr>
<td>Basal lamina and extracellular matrix</td>
<td>None</td>
<td>Neurofilament, Plectin, β amyloid 1-40, 1-42</td>
</tr>
<tr>
<td>Others</td>
<td>Caspase-3, Polyglutamine</td>
<td></td>
</tr>
</tbody>
</table>

UPR, unfolded protein response; ERAD, endoplasmic reticulum stress-associated degradation; C, central staining of RB; VCP, valosin-containing protein; P, peripheral staining of RB.

Quantitative RT-PCR. Total ribonucleic acid was extracted from frozen muscles of patient 1, one sRBM, and three age-matched controls as previously described. Quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) was performed using iCycler (Bio-Rad Laboratories, Richmond, California) adhering to the manufacturer’s protocol. Primer sequences for the 78-kDa glucose-regulated protein (GRP78) gene (F: 5′-GTGGTAGTGAAGCTGAAAGG; R: 5′-TGGAGTCTCACTCTTGTCGG) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene (F: 5′-GGTAAAGTGATATTGTGGCGATCAATG; R: 5′-GGAGGGATCTCGCTCCTGGAAGATGGTGG) were used. The values of GRP78 mRNA were normalized to that of G3PDH.

Mutation Analysis. Genomic deoxyribonucleic acid was isolated from peripheral lymphocytes using a standard technique. Sequence analysis of the valosin-containing protein (VCP) gene was directly performed using ABI PRISM 3100 automated sequencer (Applied Biosystems Japan, Tokyo, Japan). Information on primer sequence and cond-
tions of polymerase chain reaction are available upon request.

RESULTS

Immunohistochemical and Western Blot Analyses. RBs were strongly stained for the various antibodies used in the muscles from hRBM (Table 1, Fig. 1). Serial sections revealed that larger-sized RBs showed positive immunoreactions for polyubiquitin, ER chaperones, membrane-associated proteins, nuclear envelope proteins, and caspase-3. The periphery of the RBs was immunoreactive for the proteasome P27 subunit, actin, and desmin, and the center of the RBs was immunoreactive for \( \gamma \)-tubulin, a centrosome-specific tubulin. Immunoblotting analysis revealed an increased amount of

![Image](https://image-url.com)

**FIGURE 1.** Immunohistochemical features of reducing bodies (RBs, arrows) in patient 1. (A–I) and (J–R) are serial sections. RBs appear as brightly eosinophilic sarcoplasmic inclusions on hematoxylin and eosin (A,J). Various plasma membrane proteins such as dystrophin (B) and integrin \( \alpha 7 \beta 3 \) (C) as well as internal membrane protein SERCA1 (D) and nuclear membrane protein, emerin (E) are present in RBs. No immunoreactivity for extracellular matrix proteins including merosin (F) and collagen VI (G) is seen. Actin (H) and desmin (I) occasionally form a cage encircling RBs. RBs are also highlighted by antibodies against unfolded protein response-related molecules such as GRP78 (K), PDI (L), and IRE1\( \alpha \) (M), as well as ERAD-related proteins including VCP (N) and polyubiquitin (O). Immunoreactivity of p-PERK (P), an active form of PERK, confirms the activation of unfolded protein response. Proteasome (Q) stains only the periphery of reducing body while \( \gamma \)-tubulin (R) predominantly marks its center. Scale bar, 40 \( \mu m \).
GRP78 expression in muscle from patient 1 compared to the control muscle (Fig. 2A).

**Quantitative RT-PCR.** The expression of GRP78 mRNA was much higher in the muscles from both patient 1 and one sRBM than the control muscles (Fig. 2B).

**Mutation Analysis.** No mutation was identified in the VCP gene in either patient 1 or 2.

**DISCUSSION**

Recently, aggresomal features of the inclusion bodies have been reported in several neurodegenerative disorders including Huntington’s and Parkinson’s diseases. Inclusions observed in Huntington’s disease are specifically composed of mutant huntingtin together with ER chaperones and ubiquitin, whereas α-synuclein is the major component in Lewy bodies observed in Parkinson’s disease.9

In this study, we demonstrated that RBs observed in hRBM patients contained virtually all membrane-associated proteins examined including those of nuclei, sarcoplasmic reticulum, Golgi apparatus, lysosome, and plasma membrane. RBs also had aggresomal features; i.e., positive immunoreaction for ubiquitin and ER chaperones, and positive central immunoreaction for γ-tubulin, and were surrounded by desmin, a major intermediate filament protein of skeletal muscle. Furthermore, increased mRNA and protein levels of GRP78 were observed in the muscle from the hRBM patient. GRP78 is a molecular chaperone, which is upregulated during UPR. Positive immunoreaction for phosphorylated (activated) pancreatic ER kinase observed in RBs also indicates the activation of UPR. From these results, accumulation of various misfolded membrane proteins in ER could be a primary event in hRBM patients, which results in activation of the UPR and subsequent aggresome formation. γ-Tubulin is a marker of the centrosome. Although postmitotic cells like muscle fibers and neurons normally do not contain a centrosome, γ-tubulin distinctly exists in the cytosol. In neurons, the cytosolic γ-tubulins could be reorganized to form juxtanuclear condensation under ER stress, and this lesion could be the microtubule organization center.9 Positive immunoreaction for γ-tubulin in the center of RBs also suggests ER stress in hRBM.

Except for the consistency of ubiquitin immunoreactivity, previous reports showed equivocal immunohistochemical results of RBs.2 To know whether the present results could apply to other patients with RBM, we also examined muscle specimens from two sRBM patients with the severe infantile form of the disease. All RBs found in sRBM patients yielded positive immunoreactivity of GRP78, ubiquitin, and emerin, but only a subset of RBs was highlighted by dystrophin and α-sarcoglycan. A desmin-positive rim was not seen in RBs in the sRBM muscles. Deposition of the proteins associated with UPR and ER-associated degradation, together with upregulation of GRP78 mRNA also indicates the activation of UPR in sRBM muscle samples.
Recently, a mutation in the VCP gene, a key molecule in the retrotranslocation step of ER stress-associated degradation, was identified in patients with inclusion-body myopathy associated with Paget’s disease of bone and frontotemporal dementia (IBMPFD). Due to the similarity of VCP-positive inclusions observed in the hRBM to that in IBMPFD, sequence analysis of the VCP gene was performed. However, no mutation was identified in the patients with hRBM.

In conclusion, our data show the aggresomal features of RBs, which might be induced by accumulation of a battery of membrane-associated proteins, resulting in the activation of UPR. To determine the precise pathomechanism of RBM, detailed analyses on the functions of ER chaperones and proteasomes should be investigated. In animal studies, overexpression of chaperones or application of chaperone-inducing compounds such as radicicol is beneficial for the treatment of neurodegenerative diseases with inclusion bodies. Upregulation of chaperone transcription may be an option for the development of therapy in RBM.

We thank Dr. A. Kakizuka (Kyoto University, Japan) for providing the antibody for VCP, and Dr. M. Astejada (National Institute of Neuroscience, Tokyo, Japan) for reviewing the article. This work was supported by the Research on Health Sciences focusing on Drug Innovation from the Japanese Health Sciences Foundation; by the Research on Psychiatric and Neurological Diseases and Mental Health of Health and Labor Sciences research grants and the research grant (17A-10) for nervous and mental disorders from the Ministry of Health, Labor and Welfare; by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science; and by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation.
ABSTRACT: Continuous epidural stimulation of lumbar posterior root af-
ferents can modify the activity of lumbar cord networks and motoneurons,
resulting in suppression of spasticity or elicitation of locomotor-like move-
ments in spinal cord–injured people. The aim of the present study was to
demonstrate that posterior root afferents can also be depolarized by trans-
cutaneous stimulation with moderate stimulus intensities. In healthy sub-
jects, single stimuli applied through surface electrodes placed over the
T11–T12 vertebrae with a mean intensity of 28.6 V elicited simultaneous,
bilateral monosynaptic reflexes in quadriceps, hamstrings, tibialis anterior,
and triceps surae by depolarization of lumbosacral posterior root fibers. The
nature of these posterior root–muscle reflexes was demonstrated by the
duration of the refractory period, and by modifying the responses with
vibration and active and passive movements. Stimulation over the L4–L5
vertebrae selectively depolarized posterior root fibers or additionally acti-
vated anterior root fibers within the cauda equina depending on stimulus
intensity. Transcutaneous posterior root stimulation with single pulses allows
neurophysiological studies of state- and task-dependent modulations of
monosynaptic reflexes at multiple segmental levels. Continuous transcuta-
neous posterior root stimulation represents a novel, non-invasive, neuro-
modulative approach for individuals with different neurological disorders.

POSTERIOR ROOT–MUSCLE REFLEXES ELICITED
BY TRANSCUTANEOUS STIMULATION OF
THE HUMAN LUMBOSACRAL CORD

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Spinal reflex studies have led to a greater under-
standing of the monosynaptic reflex.3,7,22 Hoffmann
in 1918 reported that electrical stimulation of the
posterior tibial nerve in the popliteal fossa evoked
long-latency responses of motor units of the triceps
surae in humans.9 These reflex responses were
termed the H reflex17 and further physiologically
examined by Magladery and colleagues.18 The dem-
stration that such reflex activity could be recorded
in humans rapidly advanced the means of assessing
supraspinal and sensorimotor control in healthy in-
dividuals and those with neurological diseases.11,25

Lloyd in 1943 reported the elicitation of “dorsal
root-ventral root reflex discharges” in a cat model.
He recorded a reflex discharge from an anterior
root after single-shock stimulation of the posterior
root of the same spinal cord segment. The reflex
discharge displayed a prominent initial peak
thought to be transmitted through a two-neuron
reflex arc.14 In humans, non-invasive detection of
dorsal root-ventral root reflex discharges can be
accomplished by electromyographic recording from
the muscle to which the motoneuron discharge is
directed as a monosynaptic reflex. Such posterior
root–muscle reflexes (PRM reflexes)12,20,21 are the
basic components of the lower-limb muscle re-
sponses that are elicited by epidural stimulation of
posterior lumbar cord structures. Monosynaptic
PRM reflexes involve the same type of neurons as the
stretch reflex or H reflex of the corresponding mus-

Abbreviations: CMAP, compound muscle action potential; EMG, electro-
myography; H, hamstrings; PRM, posterior root–muscle; Q, quadriceps; TA,
tibialis anterior; TENS, transcutaneous electrical neural stimulation; TS, tri-
ceps surae
Key words: cauda equina; electrophysiology; H reflex; lumbosacral cord;
posterior roots; transcutaneous stimulation
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PRM reflexes are elicited in the same sensory axons of large-diameter afferents in the peripheral nerves, 

PRM reflexes were studied in the quadriceps, hamstrings, tibialis anterior, and triceps surae muscles.20 Whereas the H reflex is evoked by stimulation of large-diameter afferents in the peripheral nerves, PRM reflexes are elicited in the same sensory axons at proximal sites adjacent to the spinal cord. Recent studies have shown that PRM reflexes of the soleus can be elicited non-invasively in humans by high-voltage transcutaneous electrical stimulation of nerve roots at the cauda equina.16,27,30 However, PRM reflexes evoked by these methods were detected only in the soleus; the responses to the same stimulation in other muscles studied were M waves.16,27

The purpose of the present study is to describe a novel approach to elicit short-latency reflexes in muscles innervated by motoneurons from multiple segmental levels. We describe the electrophysiological characteristics of simultaneously evoked bilateral PRM reflexes in quadriceps, hamstrings, tibialis anterior, and triceps surae by transcutaneous stimulation of the posterior roots at lumbosacral cord levels. We also show that, at a more caudal site, stimulation of the cauda equina can give rise to PRM reflexes as well as to direct motor responses that are initiated in the anterior roots. Our non-invasive technique promises to open a new avenue for studies of the peripheral and central mechanisms involved in reflexes and their interaction. Preliminary results of this work have been reported elsewhere.6

MATERIALS AND METHODS

Subjects. This study was conducted on eight men, five members of our laboratory staff and three volunteers, aged 20–32 (mean, 27.6) years, with a mean height of 181 cm. Subjects were numbered according to their height, with subject 1 being the tallest. All subjects signed written informed consent to participate, and the study was approved by the local ethics committee.

Electrode and Stimulation Set-Up. Electrical stimulation was performed using commercially available self-adhesive transcutaneous electrical neural stimulation (TENS) electrodes (Schwa-medico GmbH, Ehringshausen, Germany). A pair of round electrodes with a diameter of 5 cm was placed over the paravertebral skin 1 cm apart on each side of the spine. This paravertebral electrode pair was positioned between two adjacent spinal processes at the designated rostrocaudal level. A pair of rectangular electrodes (8 cm × 13 cm each) was placed longitudinally over the abdomen, one on either side of the umbilicus. The two electrodes of each pair were connected to function as a single electrode.

A constant-voltage stimulator was used to deliver symmetric, biphasic rectangular pulses. Single stimuli with pulse widths of 2 ms and pairs of stimuli with an interstimulus interval of 50 ms were applied with intensities up to 50 V. The electrodes were connected to the stimulator such that the paravertebral electrodes acted as the anode during the first phase of the stimulus pulse, with the abdominal electrodes as cathode.

Recording Procedure. The electromyographic (EMG) activity of stimulus-evoked compound muscle action potentials (CMAPs) of left and right quadriceps, hamstrings, tibialis anterior, and triceps surae was recorded with pairs of silver–silver chloride surface electrodes. Each electrode pair was placed centrally over the corresponding muscle belly with an interelectrode distance of 3 cm, and oriented along the long axis of the muscles. The skin was slightly abraded to obtain electrode impedance below 5 kΩ. The EMG signals were amplified using Phoenix amplifiers (EMS-Handels GmbH, Korneuburg, Austria) with a gain of 502 over a bandwidth of 10–1000 Hz and digitized at 2048 Hz per channel. The data were analyzed off-line using WinDaq Waveform Browser playback software (Dataq Instruments, Akron, Ohio), and Microsoft Excel (Microsoft, Redmond, Washington) was used for calculations and graphs.

Study Protocol. The stimulation protocol was conducted with subjects in a relaxed, supine position. Muscle responses elicited by stimulation over the lumbosacral cord were studied by paravertebral electrodes at the T11–T12 interspinous space. Responses to cauda equina stimulation were tested by paravertebral electrodes at the L4–L5 interspinous space.

For a given stimulation site and intensity, three individual stimuli were triggered at 5-second intervals. Stimulation voltage was gradually increased in 1-V increments. Stimulus-evoked muscle responses were tested to determine whether they were the result of direct efferent stimulation or of reflex origin (see below).

Triceps surae H reflexes were elicited by peripheral nerve stimulation, using the same stimulator as above, adjusted to deliver biphasic rectangular stimuli with pulse widths of 2 ms to the posterior tibial nerve in the popliteal fossa through a round electrode (3-cm diameter), with a reference electrode (8 cm × 13 cm) over the patella.

Analysis of EMG Responses. Preliminary studies demonstrated that neural elements were stimulated (i.e., action potentials were elicited) at the transition
from the first to second phase of the biphasic stimulus, when the function of the paravertebral electrodes abruptly changed from anode to cathode. Therefore, response latencies were measured from the onset of the second phase of the biphasic stimulus pulse. The onset of a CMAP was defined as the first deflection from baseline that was larger than 5% of the peak-to-peak amplitude of the EMG. Pearson’s correlation coefficients comparing subject height with the latency of the responses to lumbosacral cord stimulation were calculated. We also measured the CMAP width of triceps surae responses to lumbosacral cord stimulation and to tibial nerve stimulation. The CMAP width was defined as the time between the onset of the CMAP and the moment of its last deflection from baseline larger than 5% of the peak-to-peak amplitude.

To describe the order of muscle recruitment, by transcutaneous lumbosacral cord stimulation, we identified response thresholds and calculated recruitment curves. Thresholds were defined for each muscle as the lowest-intensity stimulus for eliciting EMG responses with peak-to-peak amplitudes larger than 100 µV. A common threshold intensity was defined as the stimulus intensity needed to elicit EMG responses that were larger than 100 µV simultaneously in all of the recorded muscles in both lower limbs.

Standard recruitment curves were calculated from the data of all subjects except subject 2 (see Results) for each muscle group. The peak-to-peak EMG response amplitudes of each muscle were normalized to that at the maximal stimulus intensity. The response amplitudes from the left and right sides were averaged individually and grouped into intervals of 5% of the common threshold intensity. Within each interval, an average amplitude was calculated from the seven subjects for each muscle pair. The maximal stimulus intensity was restricted to 140% of the common threshold intensity. Stimulus intensity was never increased beyond the level that started to cause moderate discomfort to the subject (due to local contraction of the paravertebral muscles). To consider interside differences in muscle recruitment, the response asymmetry of each muscle was defined as the difference of left/right response amplitude to the mean of both sides and expressed as percentage of the mean value.

Identification of the Nature of Muscle Responses. In all subjects, pairs of stimuli with interstimulus intervals of 50 ms were applied to test the occurrence of depression of the conditioned second response.

The effect of unilateral Achilles tendon vibration on the responses to transcutaneous lumbosacral spinal cord stimulation was studied. The examined lower limb was positioned with the Achilles tendon resting on the vibrator. A pillow was put between the vibrator and the examination table to reduce mechanical propagation of the vibration. In this position, five control responses were elicited with constant stimulus intensity. The control responses were compared with five conditioned responses evoked while vibration was applied.

Passive, unilateral hip and knee flexion–extension movements were imposed by a therapist to study conditioning effects on the stimulus-evoked muscle responses. The cycle duration was approximately 2 seconds. Goniometers (Penny & Giles Biometrics, Ltd., Gwent, UK) were applied to assess the movements. Minimum hip and knee angles at the end of the flexion phase were approximately 135° and 90°, respectively. Five control responses were elicited while the therapist held the lower limb in an extended position. Then, passive movements were performed and stimulation at constant intensity was applied at 5-second intervals. The EMG amplitudes of the control and responses elicited during arbitrary segments of the flexion and extension phases were compared.

Finally, the subjects performed slight unilateral volitional contraction of the tibialis anterior (dorsiflexion) and, in separate trials, the triceps surae (plantar flexion) during electrical stimulation. Five control and conditioned responses were compared.

Statistical Analysis of the Conditioning-Test Paradigms. All unilateral conditioning–test paradigms were conducted on both sides in separate trials. Peak-to-peak amplitudes of left and right muscle responses were arranged into ipsilateral and contralateral groups (with respect to the conditioned side). For each subject and maneuver, five control and five conditioned response amplitudes were measured and averaged for each muscle. The mean conditioned response was then normalized to the control value. The normalized conditioned ipsilateral and contralateral responses resulting from the tests of both sides were averaged individually. Finally, the group results were calculated. To test the statistical significance of the differences between average conditioned and control response amplitudes, the Wilcoxon test was used, with significance set at $P < 0.05$.

RESULTS

PRM Reflexes to Transcutaneous Lumbosacral Cord Stimulation. PRM reflexes were evoked in all recorded muscles by transcutaneous electrical stimulation of the lumbosacral cord in seven of the eight
subjects. Figure 1 shows representative CMAPs in the left and right quadriceps (Q), hamstrings (H), tibialis anterior (TA), and triceps surae (TS) elicited by a single pulse. Responses to a second pulse applied after 50 ms were depressed in all muscles. In one participant (subject 2), only direct motor responses were detected in Q, whereas bilateral PRM reflexes were elicited in the other muscles. Group average values reported here were calculated only from the seven subjects in whom PRM reflexes were recorded in all muscles.

Standard recruitment curves calculated from the seven-subject group data are shown in Figure 2A, with supplementary information on relative thresholds given in Figure 2B. Graded lumbosacral cord stimulation resulted in recruitment curves for H, TA, and TS muscles that were of similar shapes (Fig. 2A). The stimulus–response relationships for these muscles followed a sigmoid curve initially with moderate slopes, growing more steeply with increasing stimulus strengths above 90% of the common threshold intensity until a plateau was reached. The Q recruitment curve demonstrated broad variations due to interindividual differences. Two characteristic curve types could be distinguished. One type, seen in three subjects, had a sigmoid shape and showed higher relative thresholds. The other type started with a steep initial slope at lower relative thresholds. The large standard deviation seen in the Q thresholds (Fig. 2B) further added to the variation across individual Q recruitment curves from the average.

Mean thresholds for PRM reflexes were: Q, 24.6 V; H, 23.4 V; TA, 26.6 V; and TS, 25.3 V. Relative values with respect to the common threshold intensity are displayed in Figure 2B. The mean common threshold intensity needed to elicit PRM reflexes bilaterally in all muscles was 28.6 ± 6.3 V in the seven-subject group. At this stimulus intensity, mean EMG amplitudes of the responses were: Q, 848.0 ± 735.9 V; H, 1683.4 ± 986.7 V; TA, 449.4 ± 356.9 V; and TS, 2686.0 ± 2060.2 V. The asymmetries amounted to: Q, 21.0 ± 26.9%; H, 18.5 ± 13.5%; TA, 43.6 ± 20.1%; and TS, 25.5 ± 23.8%.

When pairs of stimuli were applied to the lumbosacral cord at the common threshold intensity with an interstimulus interval of 50 ms, the second pulse evoked either no response or low-amplitude responses. This prolonged refractory period excluded direct electrical stimulation of efferent structures as a probable mechanism. For up to 107.8% of the common threshold intensity, all responses to the second stimulus in the pair were less than 20% of the response to the first pulse.

In one participant (subject 8), an increase of stimulus intensity to 120% of the common threshold intensity was accompanied by a discrete shortening
of response latencies by 1.4 ms. Furthermore, when a pair of stimuli was applied with 50-ms interstimulus interval, the amplitudes of the second responses were 32%-90% of the first. The shorter latencies and the absence of depression of the second response indicated direct activation of α-motoneurons in addition to the recruitment of afferent structures in this case.

Mean PRM reflex latencies at the common threshold intensity of subjects 1 and 3–8 (mean height 180.3 cm) were: Q, 10.3 ± 1.1 ms; H, 11.2 ± 0.4 ms; TA, 19.1 ± 0.9 ms; and TS, 19.7 ± 1.1 ms. Pearson’s correlation coefficients comparing latency with subject height were: Q, r = 0.95; H, r = 0.61; TA, r = 0.91; and TS, r = 0.90.

The mean latency of the triceps surae H reflex elicited by tibial nerve stimulation in the popliteal fossa was 31.3 ± 1.6 ms in the original eight subjects. The mean latency of lumbosacral spinal cord stimu-
lation–evoked TS responses was 19.8 ± 1.0 ms, thus being 63.2 ± 1.2% of the H-reflex delay, in the same subject group. The shapes of the CMAPs elicited by stimulation at the two different sites were identical and invariably had a triphasic waveform with a small initial negativity, followed by dominating positive and negative peaks. The CMAP widths of the H reflexes were 11.5 ± 2.1 ms and 13.5 ± 1.9 ms of the TS responses to spinal cord stimulation, respectively.

**Nature of the Stimulus-Evoked Muscle Responses.**

Figure 3A illustrates the conditioning effect of additional afferent input to the spinal cord produced by unilateral Achilles tendon vibration on the PRM reflexes. The responses in all ipsilateral muscles were markedly suppressed during vibration. Responses in the contralateral muscles were also reduced, although the depression was less distinct than on the ipsilateral side. These findings signify that the transcutaneous lumbosacral spinal cord stimulation–evoked muscle responses are of reflex origin.

The reflex nature of these responses was also supported by the effect of a slight volitional contraction of the leg muscles on the PRM reflex amplitudes (Fig. 3B). Slight plantar flexion significantly increased peak-to-peak amplitudes of TS responses ipsilateral to the volitional activation. Triceps surae responses elicited during voluntary contraction of the antagonistic TA were suppressed. This volitional motor task also reduced the EMG amplitudes recorded in the ipsilateral TA and H. Both volitional conditioning–test paradigms had no significant influence on the responses from contralateral H, TA, and TS, but those from the contralateral Q were facilitated.

Unilateral passive hip and knee movements significantly reduced the response amplitudes of the ipsilateral muscles during both extension and flexion phases, with the exception of the responses of the H muscle during the extension phase (Fig. 3C). In all subjects and studied muscles, the mean amplitudes of responses elicited during the flexion phase were smaller than those from the extension phase. Responses of H, TA, and TS in the contralateral lower limb that was resting in an extended position during the unilateral conditioning–test paradigm did not demonstrate significant modulations, with the exception of a reduction in the H muscle during passive flexion of the other limb. Responses in the contralateral Q were reduced throughout the passive movement of the other limb.

**Muscle Responses to Transcutaneous Cauda Equina Stimulation.** Applying graded stimulation to the cauda equina through paravertebral electrodes placed over the L4–L5 interspinous space resulted in a complex but characteristic sequence of indirect and direct responses in the lower limb muscles (Fig. 4). The average muscle recruitment order was TS first, TA second, and H third, with mean thresholds of 19.6 V, 21.0 V, and 21.2 V, respectively. At 23.6 ± 4.0 V, responses were simultaneously and bilaterally
elicited in H, TA, and TS in all subjects, whereas no responses were evoked in Q at this stimulus intensity.

The mean latencies of responses to cauda equina stimulation elicited at threshold intensities were: H, 13.3 ± 1.0 ms; TA, 21.1 ± 1.0 ms; and TS, 21.3 ± 1.1 ms, in the eight subjects. These latencies were longer than the latencies of responses to lumbosacral cord stimulation by 2.1 ms for H, 2.0 ms for TA, and 1.6 ms for TS. The longer latencies of threshold responses elicited by stimulation at the more caudal site suggests the excitation of afferent structures. The shapes of the CMAPs were identical for each muscle (except of Q) for both stimulation sites.

As the intensity of stimulation applied to the cauda equina was increased above threshold, there was initially a progressive increase in amplitudes of the H, TA, and TS responses without changes in the CMAP shapes or response latencies. Stronger stimulation produced additional, earlier EMG components that resulted in an abrupt shortening of the response latencies to: H, 9.0 ± 0.8 ms; TA, 16.4 ± 1.0 ms; and TS, 17.2 ± 1.6 ms. The short-latency EMG components increased in size with yet stronger stimulation along with a progressive decrease of the longer-latency EMG components. The mean latencies declined to: H, 7.8 ± 1.3 ms; TA, 15.5 ± 1.3 ms; and TS, 16.6 ± 2.2 ms, at the maximal stimulation intensity, suggesting activation of efferent structures at rather distal sites. The aforementioned recruitment of H, TA, and TS responses to cauda equina stimulation resembled the H-reflex and M-wave recruitment known from mixed peripheral nerve stimulation. In the Q muscle, cauda equina stimulation produced only M waves that had short latencies (mean, 6.5 ms) and generally did not exceed EMG amplitudes of 150 μV.

**DISCUSSION**

The present study provides evidence that transcutaneous stimulation can depolarize lumbosacral posterior roots, eliciting bilateral short-latency reflexes at several segmental levels. Other investigators have shown that the soleus H reflex can be elicited by
high-voltage (300–750 V) transcutaneous electrical stimulation of spinal roots.\textsuperscript{16,27,30} Responses recorded from Q, TA, extensor digitorum brevis,\textsuperscript{16} and flexor hallucis brevis\textsuperscript{27} muscles to root stimulation were reported to be M waves. This finding that a reflex response was only evoked in a single muscle may be explained by the stimulation techniques applied in the aforementioned studies. When the anatomy of the terminal spinal cord and lumbosacral roots is taken into account, there is no rational way to explain why PRM reflexes should not be evoked in several muscles by appropriate stimulation.\textsuperscript{20,21}

**Electrical Phenomena and Spinal Cord Anatomy.** Stimulating through surface electrodes as in the present study induces a current flow perpendicular to the spine\textsuperscript{27} with a high current density near the paravertebral electrodes. Some current presumably flows through the ligaments between the spinal processes and laminae of adjacent vertebrae into the vertebral canal, where a high current density will be present in the well-conducting cerebrospinal fluid.\textsuperscript{10} Such stimulation will favor spinal roots that are immersed in the cerebrospinal fluid, rather than neurons located in the spinal cord, which has a lower conductivity than the cerebrospinal fluid.\textsuperscript{10} Group Ia afferents in the posterior roots will have the lowest thresholds. They have the largest fiber diameters and are closest to the paravertebral electrodes.\textsuperscript{23} In addition, posterior root fibers have low thresholds at the sites entering the spinal cord due to their strong curvatures and the fact that they cross the interface of two media with different conductivities.\textsuperscript{15,24,26}

The arrangement of the spinal roots characteristically changes from the lumbosacral cord\textsuperscript{29} in the caudal direction.\textsuperscript{29} At the lumbosacral cord, the posterior root fibers are separated from the anteriorly located motor fibers by the spinal cord. Therefore, stimulation over the lumbosacral cord can selectively depolarize the afferent fibers due to their anatomical isolation and posterior location. The elicitation of pure PRM reflexes in the Q, H, TA, and TS of both lower limbs can be explained by the activation of group Ia muscle spindle afferents within the proximal portion of the L2–S2 posterior roots.

In the lower cauda equina, posterior root fibers associated with H, TA, and TS are close to the corresponding anterior motor bundles in the thecal sac.\textsuperscript{3} Consequently, recruitment of afferents and efferents at this caudal site by graded stimulation was similar to the results of stimulation in mixed peripheral nerves.\textsuperscript{25} By contrast, the L2–L4 spinal roots exit the thecal sac rostral to the stimulation site over the L4–L5 interspinous space. In the Q muscle, only direct motor responses of low amplitude were evoked by the lower cauda equina stimulation and were probably elicited in the corresponding anterior roots or spinal nerves at the intervertebral foramen.\textsuperscript{15}

**Evidence for the Reflex Nature of the Responses.** By stimulating the cauda equina, PRM reflexes could be distinguished from direct motor responses by their different latencies resulting from differences in length of the transmitting pathways.\textsuperscript{30} In the case of lumbosacral spinal cord stimulation, identification of the nature of the responses based on their latencies might not be definitive,\textsuperscript{8,27} but neurophysiological methods can be applied for this purpose.

Muscle responses elicited by transcutaneous lumbosacral cord stimulation at the common threshold intensity were depressed 50 ms after stimulation. This refractory period excluded the possibility that the responses were produced by direct activation of α-motoneurons in the ventral horn or anterior roots.\textsuperscript{4,26} When stimulus intensity was increased, responses of low amplitude could be evoked by the second of a pair of stimuli applied with a 50-ms interstimulus interval, presumably because the refractory period of PRM reflexes depends on the stimulus intensity.\textsuperscript{20} Further increase of stimulus intensity could result in the activation of both afferents and efferents, as was observed in one subject.

Supporting evidence for the reflex nature of the responses to lumbosacral cord stimulation was provided by the effects of tendon vibration and active and passive maneuvers. Achilles tendon vibration suppressed the ipsilateral TS responses. Vibration characteristically suppresses monosynaptic reflex pathways of the homologous muscle but not longer-latency reflex pathways.\textsuperscript{19} There was also a distinct reduction of responses in the other ipsilateral muscles, and modification of those from the contralateral side. This widespread effect of vibration could be due to a partial occlusion of input to the spinal cord carried via large afferents.

Slight voluntary contraction of TA inhibited the responses in the antagonistic TS just as voluntary dorsiflexion depresses the monosynaptic soleus H reflex to tibial nerve stimulation through the activation of group Ia inhibitory pathways.\textsuperscript{3} Voluntary contraction of TS facilitated stimulus-evoked responses in the same muscle. Unexpected results were found in the conditioned responses of TA that require further study to understand the underlying mechanisms.

Unilateral passive hip and knee movements attenuated the responses of the ipsilateral muscles.
This was a consistent finding in all subjects. The extent to which this result depended on the biomechanics or the sensory conditioning of segmental reflexes is uncertain. Regardless, these modifications support the reflex nature of the responses. The absence of modifications of responses in the H, TA, and TS muscles of the non-moved contralateral lower limb confirmed constant stimulation conditions during the passive movements.

PRM Reflexes and the H Reflex. The PRM reflex appears to be the functional equivalent of the H reflex. Both are initiated in the same type of sensory axons, except that the PRM reflex results from excitation of sensory input at proximal sites adjacent to the spinal cord. This interpretation is supported by the similar CMAP shapes and widths of the PRM reflex and H reflex of the TS, with the PRM reflex shifted to shorter latencies.

There may also be differences between the PRM reflex and the H reflex. Simultaneously evoked PRM reflexes from several adjacent segments of the spinal cord and both sides may influence each other. Facilitation from close synergists, disynaptic inhibition from antagonists, or influence from the contralateral side can affect the PRM reflexes. Due to the synchronicity of the stimulus-evoked multisegmental afferent inputs, however, short-latency effects alone may modify the excitability of central components of the PRM reflex pathways, particularly when the PRM reflex is of a mono- or oligosynaptic nature. This assumption only holds for single-pulse or low-frequency stimulation. When stimuli are applied in close succession, PRM reflexes will be conditioned by the effects of the preceding stimuli. Trains of stimuli can engage central spinal components, which influence and shape the generated motor output.

Significance of the Results. Our transcutaneous approach of posterior root stimulation is non-invasive, involves simple electrode placement and moderate stimulus intensities, and delivers stimulation at a fixed site. The widespread nature of the stimulus-evoked sensory input can be significant when exploring how the nervous system simultaneously modulates reflexes across multiple segmental levels. A potential clinical application of this method is in the assessment of the functional condition of the lumbar sacral cord and the cauda equina. Future work will show how far repetitive transcutaneous posterior root stimulation can modify the central state of excitability of lumbar cord networks, particularly in the case of decreased descending drive from brain structures due to different neurological disorders.

REFERENCES


ABSTRACT: The effects of hyperbaric exposure with high oxygen concentration on glucose and insulin levels and skeletal muscle-fiber properties were investigated in type 2 diabetic Goto-Kakizaki rats. Five-week-old rats were exposed to a hyperbaric environment (1.25 atmospheric pressure) with a high oxygen concentration (36%) for 6 h daily. Glucose and insulin levels and properties including fiber-type distribution, cross-sectional area, and oxidative enzyme activity in the soleus muscle were examined after hyperbaric exposure for 4 weeks. The growth-related increase in glucose level was inhibited by hyperbaric exposure, and insulin also showed lower levels compared with control rats. The percentage of low-oxidative type I fibers in the muscle decreased and high-oxidative type IIA and type IIC fibers, which were not detected in the muscle of control rats, were observed after hyperbaric exposure. The oxidative enzyme activity of type I fibers in the muscle increased after hyperbaric exposure. Hyperbaric exposure with high oxygen concentration might therefore provide a new approach to improve the glucose tolerance, insulin resistance, and altered skeletal muscle metabolism that are caused by diabetes mellitus.

EFFECTS OF HYPERBARIC EXPOSURE WITH HIGH OXYGEN CONCENTRATION ON GLUCOSE AND INSULIN LEVELS AND SKELETAL MUSCLE-FIBER PROPERTIES IN DIABETIC RATS

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Skeletal muscles are comprised of heterogeneous types of fibers that have different functional, morphological, and metabolic properties.7,20 Patients with type 2 diabetes mellitus have altered patterns of fiber types in the skeletal muscles, i.e., a decreased percentage of high-oxidative fibers in the skeletal muscles.5,7,22 Our previous studies29,30 revealed that Otsuka Long–Evans Tokushima Fatty (OLETF) and Goto–Kakizaki (GK) rats, animal models of spontaneous type 2 diabetes mellitus that have hyperglycemia and insulin resistance, have a lower percentage of high-oxidative fibers in the skeletal muscles than age-matched nondiabetic rats. Skeletal muscle is a major target of insulin-stimulated glucose uptake. Therefore, altered patterns of fiber types in the skeletal muscles of patients and animal models with type 2 diabetes mellitus may be linked to glucose tolerance and insulin resistance.

We have designed a hyperbaric chamber for animal experiments, which is an oxygen tank with an oxygen concentrator and an air compressor9 that automatically maintain the elevated atmospheric pressure and oxygen concentration. Increased atmospheric pressure enhances the partial pressure of oxygen and causes more oxygen to dissolve into the blood and plasma.

Our recent study28 observed that hyperbaric exposure with high oxygen concentration inhibited a growth-related increase in the glucose level of GK
rats. We postulated that the increased availability of oxygen induced by hyperbaric exposure might have a beneficial impact on the metabolism of skeletal muscles, for example, on oxidative enzyme activity, which might be related to improvements in glucose tolerance and insulin resistance. In the present study we tested this hypothesis by exposing GK rats to a hyperbaric environment with high oxygen concentration for a period of 4 weeks, and then determined glucose and insulin levels and closely examined the fiber-type distribution, cross-sectional area, and oxidative enzyme activity of fibers in the soleus muscle.

MATERIALS AND METHODS

Animals and Treatments. All procedures were approved by our institutional review committee and followed US national guidelines.

GK rats are animal models of type 2 diabetes mellitus, developed by selective breeding of an outbred colony of Wistar rats with high glucose levels as measured by the oral glucose tolerance test.4 They were selected for the present study because they have elevated levels of glucose, but not of insulin, and they do not become obese.1,11,23,24

Five-week-old male Wistar (n = 10) or GK (n = 10) rats were randomly assigned to control (n = 5) or hyperbaric (n = 5) groups. All rats were individually housed in cages of the same size. The rats in the hyperbaric group were exposed to an atmospheric pressure of 1.25 with an oxygen concentration of 36% automatically maintained by a computer-assisted system. The chamber was 180 cm long and 70 cm in diameter, making it large enough to house a number of rats (up to 20 cages) simultaneously.9 Rats in the hyperbaric group were exposed to the hyperbaric environment for 6 h (10:00 to 16:00) daily for 4 weeks. Food and water were provided ad libitum for both groups. All rats were kept in a controlled environment with fixed 12:12h light:dark cycles (lights off from 19:00 to 07:00) and room temperature maintained at 22 ± 2°C. Food intake in a 24-h period was measured.

Tissue Preparation. The rats were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The soleus muscle was removed, cleaned of excess fat and connective tissue, and wet-weighed. White adipose tissue including epididymal, omental, and retroperitoneal fat was surgically removed and weighed. The total weight of these three types of tissues was taken to be the white adipose tissue weight.

Measurements of Fasting Plasma Glucose and Insulin. Plasma obtained by centrifugation was used for measurements of glucose and immunoreactive insulin

![FIGURE 1. Transverse sections of the soleus muscle in nondiabetic Wistar rats under normobaric (A1–A3) and hyperbaric (B1–B3) conditions. (A1,B1) Stained for adenosine triphosphatase activity following preincubation at pH 10.4; (A2,B2) stained for adenosine triphosphatase activity following preincubation at pH 4.5; (A3,B3) stained for succinate dehydrogenase activity. 1, type 1; 2, type IIA; 3, type IIC. Scale bar, 100 μm.](image-url)
Plasma glucose was determined by a glucose oxidative method on blood samples obtained from the tail veins at 5, 7, and 9 weeks of age. Plasma IRI was determined by a radioimmunoassay using a polyethylene glycol method with rat plasma insulin as the standard on blood samples obtained from the abdominal aorta at 9 weeks of age.

**Histochemical Procedures.** The muscle was placed on cork, stretched to its in vivo length, and immediately frozen in isopentane cooled in a mixture of dry ice and acetone. Serial 10-μm thick transverse sections of the muscle were cut in a cryostat set at −20°C. The sections were brought to room temperature, air-dried for 30 min, then stained for adenosine triphosphatase (ATPase) activity following acid (pH 4.3 and 4.5) and alkaline (pH 10.4) preincubation (Figs. 1, 2). The muscle fibers were classified into type I (positive at preincubation pH 4.3 and 4.5, and negative at preincubation pH 10.4), type IIA (negative at preincubation pH 4.3 and 4.5, and positive at preincubation pH 10.4), and type IIC (positive at preincubation pH 4.3, 4.5, and 10.4). The fiber-type distribution of the muscle was determined from the entire transverse section of the muscle.

The sections were also stained for succinate dehydrogenase (SDH) activity, an indicator of mitochondrial capacity (Figs. 1, 2). Tissue sections were digitized as gray scale images and the value of the SDH staining intensity was expressed as an optical density (OD) value on a computer-assisted image processing system (Neuroimaging System, Kyoto, Japan). Each pixel was quantified as one of 256 gray levels. Plasma glucose was determined by a glucose oxidative method on blood samples obtained from the tail veins at 5, 7, and 9 weeks of age. Plasma IRI was determined by a radioimmunoassay using a polyethylene glycol method with rat plasma insulin as the standard on blood samples obtained from the abdominal aorta at 9 weeks of age.

**FIGURE 2.** Transverse sections of the soleus muscle in diabetic Goto-Kakizaki rats under normobaric (A1–A3) and hyperbaric (B1–B3,C1–C3) conditions. (A1,B1,C1) Stained for adenosine triphosphatase activity following preincubation at pH 10.4; (A2,B2,C2) stained for adenosine triphosphatase activity following preincubation at pH 4.5; (A3,B3,C3) stained for succinate dehydrogenase activity. 1, type I; 2, type IIA, 3, type IIC. Scale bar, 100 μm. All fibers in the muscle of GK rats under normobaric conditions were type I (A1–A3). Two of five GK rats under hyperbaric conditions had only type I fibers in the muscle, whereas two GK rats had type I and type IIC fibers (B1–B3). One rat under hyperbaric conditions had type I and type IIA fibers in the muscle (C1–C3).
levels. A gray value of zero was equivalent to 100% transmission of light, and that of 255 was equivalent to 0% transmission of light. The OD units of all pixels within the muscle fiber were converted to a mean OD unit using a calibration photographic tablet, which has 21-step gradient density ranges of diffused density values.

**Statistics.** Means and standard deviations were calculated from individual values using standard procedures. Student’s *t*-test was used to determine significant differences between the control and hyperbaric groups.

**RESULTS**

**Body Weight and Food Intake.** The body weights of Wistar rats at 9 weeks of age in the control and hyperbaric groups were 365.8 ± 24.6 g and 364.6 ± 19.2 g, respectively, and those of GK rats at 9 weeks of age in the control and hyperbaric groups were 217.4 ± 14.2 g and 206.8 ± 4.5 g (*n* = 5 for all groups), respectively. There was no difference in body weight of Wistar or GK rats between the control and hyperbaric groups.

The food intakes of Wistar rats at 9 weeks of age in the control and hyperbaric groups were 30.8 ± 3.8 g/day and 29.0 ± 3.3 g/day, respectively, and those of GK rats at 9 weeks of age in the control and hyperbaric groups were 9.5 ± 1.1 g/day and 9.0 ± 1.2 g/day (*n* = 5 for all groups), respectively. Wistar or GK rats in the control and hyperbaric groups had equivalent levels of food intake.

**White Adipose Tissue Weight.** The white adipose tissue weights of Wistar rats in the control and hyperbaric groups were 7.07 ± 2.89 g and 7.16 ± 1.28 g, respectively, and those of GK rats in the control and hyperbaric groups were 3.53 ± 0.69 g and 3.85 ± 0.57 g (*n* = 5 for all groups), respectively. There was no difference in white adipose tissue weight of Wistar or GK rats between the control and hyperbaric groups.

**Fasting Plasma Glucose and Insulin Levels.** The fasting plasma glucose levels of Wistar and GK rats were significantly lower in the hyperbaric groups at 7 and 9 weeks of age than in the control groups (Fig. 3).

The fasting plasma IRI levels of Wistar rats at 9 weeks of age in the control and hyperbaric groups were 1707.6 ± 526.7 pg/ml and 1514.5 ± 631.6 pg/ml, respectively, and those of GK rats at 9 weeks of age in the control and hyperbaric groups were 472.1 ± 238.0 pg/ml and 123.2 ± 41.3 pg/ml (*n* = 5 for all groups), respectively. The fasting plasma IRI levels of GK rats were significantly lower (*P* < 0.05) in the hyperbaric than control group, but there was no difference in fasting plasma IRI level of Wistar rats between the control and hyperbaric groups.

**Soleus Muscle Weight.** The muscle weights of Wistar rats in the control and hyperbaric groups were 0.13 ± 0.02 g and 0.13 ± 0.02 g, and those of GK rats in the control and hyperbaric groups were 0.08 ± 0.01 g and 0.09 ± 0.01 g (*n* = 5 for all groups).
FIGURE 4. Fiber-type distributions, cross-sectional areas, and succinate dehydrogenase activities of the soleus muscle in nondiabetic Wistar (WR) and diabetic Goto–Kakizaki (GK) rats. SDH, succinate dehydrogenase; OD, optical density. Values are expressed as mean ± standard deviation \([n = 5, \text{except for type IIA} (n = 1) \text{and type IIC} (n = 2) \text{in diabetic GK rats of the hyperbaric group}]. *P < 0.05, **P < 0.01 \text{compared with control value}.
respectively. There was no difference in muscle weight of Wistar or GK rats between the control and hyperbaric groups.

**Soleus Muscle Fiber Properties.** In Wistar rats there was no difference in fiber-type distribution or cross-sectional area between the control and hyperbaric groups (Fig. 4). The oxidative enzyme activities of all types of fibers were significantly higher in the hyperbaric than control group (Fig. 4).

In GK rats all fibers in the muscles of the control group were type I (Figs. 2, 4). The muscles of two rats in the hyperbaric group were composed of only type I fibers (Fig. 4). The muscles of two other rats in the hyperbaric group were composed of type I (94.0% and 92.5%) and type IIC (6.0% and 7.5%) fibers, whereas that of the other rat in this group contained type I (87.7%) and type IIA (12.3%) fibers. The cross-sectional area of type I fibers in the muscle was significantly smaller in the hyperbaric than control group, whereas the oxidative enzyme activity of type I fibers was significantly higher in the hyperbaric than control group (Fig. 4).

**DISCUSSION**

Skeletal muscle plays an important role in the regulation of blood glucose because it is the site with the highest level of insulin-stimulated glucose uptake and disposal. It is largely accepted that type 2 diabetes mellitus is associated with impaired insulin-stimulated glucose disposal capacity, which is attributed to insulin resistance in skeletal muscle. Patients with type 2 diabetes mellitus have disrupted metabolic potentials and different patterns of fiber types in the skeletal muscles compared with nondiabetic subjects. Diabetes has been associated with a high percentage of low-oxidative fibers (particularly type IIB fibers) and a low percentage of high-oxidative fibers in the fast skeletal muscles, such as the biceps femoris, vastus lateralis, and rectus abdominis muscles. We observed similar changes in the fiber-type distribution of both the fast plantaris and slow soleus muscles in diabetic rats. Previous studies suggested that a decreased percentage of high-oxidative fibers in the skeletal muscles combined with a reduction in glucose transporter (GLUT)-4 expression in high-oxidative fibers reduces the insulin-sensitive GLUT-4 pool in patients with type 2 diabetes mellitus and contributes to skeletal muscle insulin resistance. These results strongly indicate that changes in the fiber-type distribution of skeletal muscles in diabetic rats are due to an impairment in insulin sensitivity and glucose metabolism.

Consistent with our hypothesis that the increased availability of oxygen induced by hyperbaric exposure with high oxygen concentration has a beneficial impact on glucose and insulin levels and the metabolism of skeletal muscles, we observed that a growth-related increase in glucose level of GK rats was completely inhibited by hyperbaric exposure (Fig. 3). These findings are consistent with those in our recent study. In addition, the insulin level was significantly lower in the hyperbaric than control group. Hyperbaric exposure with high oxygen concentration might therefore provide a new approach to improve glucose tolerance and insulin resistance.

In the present study, we examined the soleus muscle because it has a higher percentage of high-oxidative fibers, which are more insulin sensitive and responsive than low-oxidative fibers. In addition, high-oxidative fibers are characterized by increased fatty acid oxidation, low glycolytic capacity, and high triglyceride accumulation compared with low-oxidative fibers. A previous study also observed that skeletal muscle insulin resistance in GK rats is associated with high-oxidative fiber-specific defects in the insulin-signal transduction pathway to glucose transport, suggesting that hyperglycemia affects high-oxidative fibers more severely than low-oxidative fibers, and that it selectively reduces GLUT-4 expression in high-oxidative fibers in patients and animal models with type 2 diabetes mellitus. In the present study, hyperbaric exposure with high oxygen concentration prevented diabetes-associated changes in the fiber-type distribution of the soleus muscle in GK rats. In addition, we observed that the type I fiber oxidative enzyme activity of the soleus muscle in GK rats increased after hyperbaric exposure. These results suggest that the increase in oxidative capacity of skeletal muscles is an adaptive response to hyperbaric exposure with high oxygen concentration.

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ABSTRACT: The purpose of this study was to document the ultrasonographic measurement differences in median nerve size between patients with carpal tunnel syndrome (CTS) and controls, and to correlate these findings with electrophysiological stage and motor unit number estimation (MUNE), thereby allowing us to test the validity of ultrasound as a diagnostic modality for assessing the severity of CTS. High-resolution sonography and electrophysiological studies were performed on 41 wrists of 27 patients and compared with findings on 40 wrists of 20 healthy individuals. On ultrasonographic views, cross-sectional area and flattening ratio in proximal, middle, and distal tunnel segments of the median nerve were measured both by calculating ellipsoid area by large and small cross-sectional diameters and by automated ellipsoid area calculation. We compared electrophysiological stage and MUNE with proximal, middle, and distal cross-sectional area and other ultrasonographic findings. All correlations between electrophysiological stage and cross-sectional areas in these different segments of the median nerve were significant with both measurement methods. Negative correlations were seen between MUNE and cross-sectional area in the proximal and middle segments, whereas no significant correlation was detected in the distal segment. Our results indicate that there are close correlations between the ultrasonographic findings and electrophysiological stage. Ultrasound also reflects the reduction in the number of axons estimated by the MUNE method. Therefore, we suggest that the ultrasonographic findings reflect the severity of disease in patients with CTS.


ULTRASONOGRAPHY IN CARPAL TUNNEL SYNDROME:
COMPARISON WITH ELECTROPHYSIOLOGICAL STAGE
AND MOTOR UNIT NUMBER ESTIMATE

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The diagnosis of carpal tunnel syndrome (CTS) is based on history, clinical findings, and electrophysiological studies. Sonography with probes working at high frequencies has proven to be useful in patients with CTS.3–5,13 Sonographic signs have been described in CTS patients and compared with electrophysiological measurements.3,11,20,21,33,34 Diffuse or localized swelling of the median nerve due to edema and flattening by compression usually causes most of the radiological findings.4,5,9,18,19 The pathophysiology of CTS is demyelination in early stages, accompanied by axonal loss in advanced stages.25 The reduction in number of axons can be estimated by the motor unit number estimation (MUNE) method.17

In this study we analyzed the sonographic finding of CTS confirmed electrophysiologically and evaluate its correlations with MUNE and electrophysiological stage in order to test the validity of ultrasound for assessing the severity of CTS.

PATIENTS AND METHODS

Patients and Controls. Between August 2004 and January 2005, we examined 41 wrists of 27 patients (6 men, 21 women) with a clinical diagnosis of CTS. Their mean age was 43.6 (range, 22–61) years. For the clinical diagnosis of CTS, patients were questioned about
primary symptoms (paresthesias, pain, clumsiness, nocturnal symptoms) in the median nerve distribution. If they had only one of these symptoms, we looked for at least two secondary symptoms (burning/cold, tightness, pain/discomfort, swelling). The mean duration of symptoms was 14 months (range, 2–72 months). The clinical diagnosis was confirmed electrophysiologically by abnormality of median nerve action potential latency in the palm–wrist segment. Where the routine nerve conduction studies (NCSs) were normal, the required factors were a median–ulnar sensory latency difference ≥0.5 ms and a median–ulnar distal motor latency difference ≥1.2 ms.

Twenty asymptomatic volunteers (7 men, 13 women) formed the control group. Mean age was 37.5 (range, 21–60) years. The age and gender difference between the groups was not significant. Electrophysiological and ultrasonographic studies were performed on both patients and healthy individuals. All participants provided informed consent.

Electrophysiological Studies. Nerve conduction studies (NCSs) and MUNE recordings were performed by the same neurologist with Keypoint EMG equipment (Medtronic, Skovlunde, Denmark). Motor and sensory conduction studies for median and ulnar nerves were performed using the standard techniques of supramaximal percutaneous stimulation. The results of NCSs were expressed as Z scores in comparison with reference values; the Z score is calculated by dividing the difference between the obtained value and expected value (based on the reference material) by the standard deviation of the reference material. The NCS results were corrected for age and height according to reference values. While expressing NCS parameters, Z < −2 was considered abnormal for conduction velocities, amplitude of sensory nerve action potentials (SNAPs), and amplitude of compound muscle action potentials (CMAPs). Z > +2 was considered abnormal for the latencies of SNAPs and CMAPs.

The hands were categorized into 5 stages according to the results of electrophysiological studies: (1) minimal CTS, abnormal comparative or segmental tests; (2) mild CTS, slowing of median digit–wrist segment and normal distal motor latency (DML); (3) moderate CTS, slowing of median digit–wrist segment and abnormal DML; (4) severe CTS, absence of median SNAPs (digit–wrist segment) and abnormal DML; and (5) extreme CTS, absence of thenar motor (and sensory) response.

MUNE was performed on the abductor pollicis brevis (APB) muscle (using the incremental technique) as described by McComas. The recording electrode was placed over the motor point of the APB muscle, with the reference over the distal interphalangeal joint, while the median nerve was stimulate at the wrist by fixed surface electrodes. After a maximal CMAP was obtained, we gradually increased the stimulus intensity from subthreshold levels until a quantal response was obtained and 11 increments were recorded. Using a computer program, the amplitude of the resultant response was divided by the number of increments and this value was divided into the maximum CMAP to give MUNE.

Ultrasonographic Studies. Ultrasonography was performed with real-time equipment (PowerVision 7000 SSA-380A; Toshiba Corporation, Tokyo, Japan) and a 10-MHz linear probe. The hands to be studied were placed on a hard surface in a neutral position. The median nerve was located in the axial section, just beneath the flexor retinaculum. Axial images of the nerve were obtained at the radial–ulnar articulation (level 1) and at the level of the pisiform (level 2) and hook of hamate (level 3); large and small cross-sectional diameters at each level were measured. Using these measurements, flattening ratio and cross-sectional area were calculated (area was assumed an ellipsoid and determined by the formula: \( r_1 \times r_2 \times 3.14 \). We also used an automated ellipsoid area measurement method by placing the ellipsoid cursor on the median nerve cross-section. The displacement of the area from the palmar apex of the retinaculum to a straight line drawn between the tubercle of trapezium and hook of hamate. Displacements were measured twice, and mean distance was accepted as palmar displacement. The interval between ultrasonography and the electrophysiological studies was approximately 1 week. All the sonographic imaging was performed by the same radiologist, who was not aware of the electrophysiological findings.

Statistical Analysis. Pearson and Spearman correlation tests were used to compare automatically measured cross-sectional area, calculated cross-sectional area, anteroposterior diameter, mediolateral diameter, flattening ratio (anteroposterior diameter/mediolateral diameter) at three levels, and mean palmar displacement with MUNE and electrophysiological stage. To compare the results in both groups, t tests were done.

RESULTS

Based on the electrophysiological evaluation, the stage of CTS was minimal in 8 hands, mild in 6, moderate in 10, severe in 16, and extreme in 1.
Ultrasoundographic measurements and MUNE in the patient and control groups are summarized in Table 1. Cross-sectional area, anteroposterior diameter, mediolateral diameter, anteroposterior diameter/mediolateral diameter, calculated cross-sectional area at the three levels, mean palmar displacement, and mean MUNE values showed significant differences between the two groups.

In the correlative study of sonographic signs of CTS with electrophysiologic stage, we observed a significant correlation for cross-sectional area in proximal, middle, and distal segments of the carpal median nerve ($r = 0.408, P < 0.01$; $r = 0.562, P < 0.001$; and $r = 0.522, P < 0.001$, respectively). Negative correlations with MUNE and cross-sectional area in the proximal and middle segments were observed ($r = -0.414, P = 0.008$; and $r = -0.324, P = 0.041$, respectively), whereas in the distal segment of the carpal median nerve no significant correlations were obtained ($r = -0.279, P = 0.082$). Most of the measurements in level 1 and level 2 correlated with electrophysiologic stage and MUNE. Although we observed a significant correlation for most of the measurements in the distal segment of the median nerve (level 3) with electrophysiologic stage, no correlations with MUNE were found in this segment (Table 2).

**DISCUSSION**

Response to compression of the median nerve is characterized by endoneurial edema, demyelination, inflammation, distal axonal degeneration, fibrosis, growth of new axons, remyelination, and thickening of the perineurium and endoneurium. Enlargement of the nerve reflects all these processes and occurs proximal to the entrapment site. These changes cause the increased cross-sectional area as detected by sonography. In patients with CTS, quantitative ultrasound findings may be useful when electrophysiological findings are negative, especially in the early stage of entrapment. More pronounced thickening of entrapped nerves at the time of diagnosis is associated with poor outcome at follow-up, whereas electrodagnostic signs of demyelination suggest a favorable outcome; Beekman et al. described the relationship between enlargement and the presence of axonal damage of the ulnar nerve.

The ultrasonographic criteria for CTS include proximal median nerve swelling, bowing of the flexor retinaculum, distal nerve flattening, and increased nerve area. In most studies, median nerve measurements were performed at a single level, mostly at the proximal carpal tunnel. Buchberger et al. performed measurements of the...
median nerve at three levels, which allows for a more complete morphological assessment of the enlarged nerve.

Nakamicki et al. discussed the correlation between severity of CTS and cross-sectional areas in four segments of the median nerve, finding a good correlation between the area within the proximal three segments and electrophysiological parameters, such as median distal motor nerve latency and median sensory nerve conduction velocity.\(^{21}\) Correlating electrophysiological grading with ultrasonographic measurements at three different levels, we observed a significant correlation for cross-sectional area, calculated cross-sectional area, and mediolateral diameter at all levels. Mean palmar displacement also correlated well with electrophysiological stage (Table 2). For all these measurements, we found significant differences between patient and control groups (Table 1). The area calculation when using anteroposterior and mediolateral diameters in the ellipsoid formula or by drawing the margins of the median nerve with electronic calipers takes more time. We believe that automated ellipsoid area calculation is the fastest approach for cross-sectional area calculation in the median nerve and shows a good correlation with calculated cross-sectional area in both patient and control groups.

We found a reduction in axon count in CTS by the MUNE method.\(^{2,7,17}\) This method has theoretical advantages over traditional motor nerve conduction studies or electromyography. Acute motor unit loss results in reduced amplitude of the CMAP, but with chronic lesions there is compensatory collateral sprouting of surviving axons, resulting in relative preservation of the amplitude. MUNE gives useful clinical information when collateral sprouting has maintained the amplitude of the CMAP.\(^{8,29}\) Correlating MUNE with ultrasonographic measurements will improve diagnostic value of ultrasound in CTS.

Although MUNE values of the control group were lower than those reported in previous studies,\(^{10,16}\) there was a significant difference between the CTS and control groups. Age-related changes may affect the validity of MUNE in elderly subjects.\(^{30}\) Our investigation did not include controls and patients >62 years of age, which is a limitation of the study.

MUNE has a negative correlation with electrophysiological stage (Tables 1 and 2). In this study we found a negative correlation between MUNE and cross-sectional or calculated cross-sectional area in the proximal (level 1) and middle (level 2) segments. The mean cross-sectional area is best obtained at these two levels because of clear visualiza-
tion. Also, these are the levels of the proximal carpal tunnel where maximum nerve swelling is expected. At level 3 no significant correlation with MUNE was noted.

In this study we found close correlations between the ultrasonographic findings and MUNE as well as electrophysiological stage. We therefore suggest that the ultrasonographic findings reflect the severity of the disease in patients with diagnosis of CTS.

REFERENCES

ABSTRACT: Intrinsic laryngeal muscles share many anatomical and physiological properties with extraocular muscles, which are unaffected in both Duchenne muscular dystrophy and mdx mice. We hypothesized that intrinsic laryngeal muscles are spared from myonecrosis in mdx mice and may serve as an additional tool to understand the mechanisms of muscle sparing in dystrophinopathy. Intrinsic laryngeal muscles and tibialis anterior (TA) muscle of adult and aged mdx and control C57Bl/10 mice were investigated. The percentage of central nucleated fibers, as a sign of muscle fibers that had undergone injury and regeneration, and myofiber labeling with Evans blue dye, as a marker of myofiber damage, were studied. Except for the cricothyroid muscle, none of the intrinsic laryngeal muscles from adult and old mdx mice showed signs of myofiber damage or Evans blue dye labeling, and all appeared to be normal. Central nucleation was readily visible in the TA of the same mdx mice. A significant increase in the percentage of central nucleated fibers was observed in adult cricothyroid muscle compared to the other intrinsic laryngeal muscles, which worsened with age. Thus, we have shown that the intrinsic laryngeal muscles are spared from the lack of dystrophin and may serve as a useful model to study the mechanisms of muscle sparing in dystrophinopathy.


INTRINSIC LARYNGEAL MUSCLES ARE SPARED FROM MYONECROSIS IN THE mdx MOUSE MODEL OF DUCHENNE MUSCULAR DYSTrophy

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Duchenne muscular dystrophy (DMD) is a severe X-linked recessive disorder characterized by progressive loss of muscular strength that affects 1 in 3500 live male births.9,10 DMD is caused by a lack of dystrophin, a cytoskeletal protein located on the inner surface of the muscle cell membrane. Lack of dystrophin predisposes the cell membrane to breakdown, leading to muscle-fiber necrosis.13 In the mdx mouse, an experimental model for DMD, skeletal muscle fibers exhibit a drastic reduction in the expression of dystrophin, which results in myonecrosis.2,6,26

The extraocular muscles (EOMs) of both DMD patients and mdx mice remain unaffected during the course of the disease. Because necrosis of muscle fibers is central in the pathophysiology of DMD, understanding the mechanisms that allow EOMs to escape from myonecrosis is of interest and has been examined extensively in mdx mice.1,3,5,16,20,22–24,27

The intrinsic laryngeal muscles (ILMs) share many anatomical and physiological properties with the EOMs.2,7,11,14 The ILMs are innervated by cranial nerves, express extraocular myosin heavy chain, and present short contraction times and continuous muscle-fiber remodeling.11,14,25 Hence, we hypothesized that the ILMs are spared from myonecrosis in the mdx mouse model of DMD and may serve as an additional tool to study the mechanisms of muscle sparing in dystrophinopathy. The present study was undertaken to investigate this hypothesis.

MATERIALS AND METHODS

Male mdx and C57Bl/10 mice obtained from the mouse breeding colony at our institution were housed under controlled conditions of 12/12-h light/dark cycle and temperature, with free access to food and water. All experiments were performed in
For visualization of muscle-fiber damage, adult (4 months of age) *mdx* (*n* = 5) and C57Bl/10 (control; *n* = 5) and old (18 months of age) *mdx* (*n* = 5) and C57Bl/10 (*n* = 5) mice were injected with Evans blue dye (EBD; Sigma, St. Louis, Missouri), a marker of sarcolemmal lesions. The animals received an intra-peritoneal injection of 1% EBD in phosphate-buffered saline (PBS) at a dose of 100 µl per 10 g body weight. Twenty-four hours later, the mice were killed with an overdose of chloral hydrate and the larynx and right tibialis anterior (TA) muscle were dissected out and snap frozen in isopentane cooled in liquid nitrogen.

Cryostat cross-sections of the larynx (transverse and longitudinal 7-µm-thick sections) and TA (trans-
verse 7-μm-thick sections) were stained with hematoxylin–eosin (H&E) for quantification of the total number of fibers and the number of fibers with central nucleation, indicative of muscle regeneration. The number of fibers and of central nucleated fibers was counted by a blinded observer. The ILMs studied were the lateral thyroarytenoid, medial thyroarytenoid (vocalis muscle), lateral cricoarytenoid, posterior cricoarytenoid, and cricothyroid (CT).

Some sections were labeled for dystrophin. Sections were air dried, hydrated for 30 min with PBS, incubated with 0.3% Triton X-100 for 10 min, and then blocked with blocking solution (15% glycine, 3% bovine serum albumin, and 0.6% Triton X-100 in PBS; Sigma) for 3 h. The sections were incubated with dystrophin antibody (NCL-DYS1 mouse monoclonal, Novacastra, Newcastle upon Tyne, UK) at 1:500 overnight at 4°C, washed with PBS, and incubated with secondary anti-mouse immunoglobulin G–fluorescein isothiocyanate (IgG-FITC; Sigma, St. Louis, Missouri) at 1:500 for 1 h at room temperature. Sections were washed again in PBS, coverslipped with 1,4-diazabicyclo[2.2.2]octane (DABCO; Sigma) mounting medium, and observed under a confocal microscope (MRC 1024; BioRad Laboratories, Hercules, California).

EBD staining appears as a bright red emission under a fluorescence microscope. Fiber counts of EBD-positive muscle fibers and H&E observation were done for all sections and photographed under a Nikon fluorescence microscope connected to a Hamamatsu video camera. Statistical analysis was performed using the ProcGLM (general linear models) of the SAS statistical program; mean comparisons were done using the average multiple comparison test (SAS Institute, Cary, North Carolina).

RESULTS

In adult and old mdx mice, no signs of myofiber damage were observed in any of the ILMs (Fig. 1A, C), except for the CT muscle (Fig. 1E). The lateral thyroarytenoid, medial thyroarytenoid (vocalis muscle), lateral CT, and posterior CT appeared to be normal, with muscle fibers round or roughly polygonal with rounded angles. In cross-sections, their nuclei were randomly placed, always found in a peripheral location directly under the sarcolemma,

<p>| Table 1. Percentage of central nucleated fibers in lateral cricoarytenoid (LCA), posterior cricoarytenoid (PCA), lateral thyroarytenoid (LTA), medial thyroarytenoid (MTA), cricothyroid (CT) and tibialis anterior muscles from adult (4-month-old) and aged (18-month-old) C57B1/10 (control) and mdx mice. |</p>
<table>
<thead>
<tr>
<th>LCA</th>
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Values represent the mean ± standard deviation; n = number of muscles examined.
*P < 0.05: significantly different from adult animals of the same strain (Duncan’s multiple comparisons of means).
†P < 0.05: significantly different from all groups (Duncan’s multiple comparisons of means).

![FIGURE 2. Transverse sections of Evans blue dye (EBD)–labeled muscles. Adult mdx lateral thyroarytenoid muscle (A) showing no EBD-positive fibers. EBD-positive fibers were observed in adult mdx cricothyroid (B) (asterisk) and tibialis anterior (C) (asterisk) muscles. Scale bar. (A) 166 μm; (B) 144 μm; (C) 183 μm.](image-url)
similar to control muscles (Fig. 1B, D). Muscle fibers had a relatively uniform diameter, and no degenerating myofibers or extensive areas of inflammatory reaction were observed (Fig. 1A, C). In these muscles, the percentage of central nucleated fibers, the morphological indicator of fibers having undergone damage, did not differ from control (Table 1). No myofibers containing EBD, an earlier marker of sarcolemmal disruption, were seen (Fig. 2A).

The adult \textit{mdx} CT muscle displayed evidence of myopathy, represented by an increased percentage of central nucleated fibers when compared to the other ILMs (Fig. 1E). Compared to the TA muscle, this increase was not significant. The dystrophic phenotype of the CT was more evident in aged mice, which showed a twofold increase in the percentage of central nucleated fibers (Table 1). Both inflammatory reaction and fiber injury, as demonstrated by EBD-positive fibers, were observed more frequently (Fig. 2B). In the TA of adult and old \textit{mdx} mice, EBD-positive fibers were present (Fig. 2C) and central nucleated fibers were readily visible (Fig. 1G and Table 1).

Control posterior CT muscles exhibited a normal pattern of dystrophin distribution, with dystrophin labeling associated with the sarcolemma (Fig. 3A). In \textit{mdx} mice, posterior CT muscles were negative for dystrophin (Fig. 3B). This shows that, despite the lack of dystrophin, there was no muscle-fiber degeneration in ILMs.

**DISCUSSION**

In the present study, we evaluated whether ILMs from \textit{mdx} mice show signs of muscle-fiber degeneration. Usually, myonecrosis in \textit{mdx} mice starts at about 3–4 weeks of age, with degeneration of limb muscles having occurred by 10 weeks of age.\(^8,26\) Except for the CT muscles, we did not observe any signs of muscle-fiber damage in the ILMs of adult or aged \textit{mdx} mice. Central nucleation was significantly lower in ILMs than in the TA muscles, as is described also for the EOMs, where myonecrosis is not observed.\(^1,3,16,22–24,27\) Therefore, the ILMs do not exhibit the pattern of muscle necrosis and regeneration seen in most \textit{mdx} skeletal muscles, demonstrating that these muscles are protected from the lack of dystrophin.

Loss of calcium homeostasis has been suggested to play a role in the mechanism of muscle necrosis in DMD and \textit{mdx} mice.\(^16\) In the EOMs, proteins involved in calcium reuptake, such as parvalbumin and sarcoplasmic reticulum calcium ATPase, are increased, and this may explain their escape from myonecrosis.\(^7\) Preliminary observations have shown that calcium reuptake and release systems are both amplified in laryngeal muscles,\(^4\) suggesting that, similar to the EOMs, dystrophic laryngeal muscles may be spared from myonecrosis by a better capacity to maintain calcium homeostasis.

Continuous myofiber remodeling has been reported in non-dystrophic EOMs as a result of fusion of satellite cells into existing myofibers, and this may account for the sparing of dystrophic EOMs in Duchenne dystrophy.\(^20\) Continuous remodeling of muscle fibers has also been reported for non-dystrophic ILMs,\(^11\) and this may explain the lack of muscle degeneration in these muscles.

We found that the CT muscle in \textit{mdx} mice is affected significantly compared to the other laryngeal muscles. The EOMs also show non-spared mus-
Intrinsic Laryngeal Muscle Sparing in mdx Mice

In conclusion, we have shown that ILMs are protected from the lack of dystrophin in adult and old mdx mice, whereas no sparing occurs of the CT muscle. Further studies of dystrophic laryngeal muscles will be needed to better understand the mechanisms of sparing and its relation to aging, and also to develop new therapeutic strategies for the treatment of dystrophinopathies.

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grants 95/6110-2, 01/00570-4, and 04/15526-9). H.S.N. and M.J.M. are recipients of fellowships from the Conselho Nacional de Pesquisas (CNPq: 302880/2004-6 and 301286/2003-5).

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ABSTRACT: We tested the hypothesis that physical activity modifies the course of age-related motor decline. More than 850 older participants of the Rush Memory and Aging Project underwent baseline assessment of physical activity and annual motor testing for up to 8 years. Nine strength measures and nine motor performance measures were summarized into composite measures of motor function. In generalized estimating equation models, global motor function declined during follow-up (estimate, −0.072; SE, 0.008; P < 0.001). Each additional hour of physical activity at baseline was associated with about a 5% decrease in the rate of global motor function decline (estimate, 0.004; SE, 0.001; P = 0.007). Secondary analyses suggested that the association of physical activity with motor decline was mostly due to the effect of physical activity on the rate of motor performance decline. Thus, higher levels of physical activity are associated with a slower rate of motor decline in older persons.

PHYSICAL ACTIVITY AND MOTOR DECLINE IN OLDER PERSONS

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Declining motor function, including decreased motor performance and muscle strength, are familiar consequences of aging and are associated with a variety of adverse health outcomes including mortality, disability, and institutionalization.1,2,14,15,36 Physical activity is a modifiable risk factor for overall musculoskeletal fitness (i.e., muscle strength, endurance, power, and flexibility) as well as cardiovascular disease and a variety of other chronic diseases.41 Previous studies have shown that a higher level of physical activity at one point in time predicts a higher level of motor function years later. However, the extent to which this association is due to a higher level of motor function at baseline is unclear because it is difficult to fully assess change in motor function as an outcome and distinguish it from initial level of performance on the basis of only two observations.8,22,36 Thus, previous studies would be complemented by a study with repeated motor measures over several years of follow-up. In addition, motor function is not a unitary process but is derived from dissociable systems that control different aspects of movement.10,16 Motor performance reflects the function of a large number of cortical and subcortical structures necessary for the planning and execution of movements,16 whereas muscle strength may predominantly reflect motor unit and muscle function.10,11,30 Consequently, the association of physical activity with motor performance and muscle strength may vary. There are no longitudinal data showing the extent to which the level of physical activity is associated with the rate of change in both motor performance measures and muscle strength.

We used data from 876 older participants of the Rush Memory and Aging Project, a longitudinal clinical-pathologic investigation of common chronic conditions of old age, who underwent detailed examinations annually for up to 8 years to examine the relation of physical activity to change in motor function.5 Motor testing included nine motor perfor-
ances and nine strength measures that were summarized into a composite measure of global motor function and separate measures of motor performance and appendicular muscle strength. We examined the relation of physical activity with change in these motor measures, controlling for several potentially confounding variables including body composition, chronic diseases, and disability.

**MATERIALS AND METHODS**

**Participants.** Participants were recruited from about 40 diverse retirement facilities and subsidized housing facilities, as well as from church groups and social service agencies in and around Chicago. Following a presentation about the study, persons rated their interest in participating. Study personnel subsequently met with those who expressed interest and explained the project in detail. Persons then signed an informed consent agreeing to annual clinical evaluation and an anatomic gift act agreeing to donate their brain, spinal cord, selected nerves, and muscles to investigators. The study was in accordance with the latest version of the Declaration of Helsinki and was approved by our institutional review board. The clinical evaluation was uniform and included a medical history, complete neurological examination, and assessment of cognitive and motor function. Follow-up evaluations, identical in all essential details, were performed annually by examiners blinded to previously collected data.

At the time of these analyses, 1,100 participants had completed a baseline evaluation. Eligibility for these analyses required the absence of clinical dementia at the baseline evaluation, a valid motor assessment at baseline, and at least one follow-up motor evaluation in order to assess change in motor function (muscle strength and motor performance). Therefore, we excluded 66 persons who met criteria for dementia at the baseline for these analyses, 143 persons who had completed a baseline evaluation but died before their first follow-up examination or had not been in the study long enough for follow-up evaluation, and 15 persons with incomplete data. This resulted in a final group of 876 nondemented persons who had one or more follow-up evaluations, with a mean follow-up of about 4 years (mean, 3.97 years; SD, 1.97). Their mean age at baseline was 80.5 years (SD, 6.9), the mean education was 14.5 years (SD, 3.0), and the mean Mini-Mental Status Examination score was 27 (SD, 2.1); 74.4% were women and 97.9% were white and non-Hispanic (see Table 1 for additional details).

**Clinical Diagnoses.** Clinical diagnoses were made using a multistep process, as previously described. First, participants underwent detailed cognitive function testing that included 21 performance tests administered in an approximately hour-long session. Detailed information about the cognitive function testing is published elsewhere. Second, the cognitive test data were reviewed by an experienced neuropsychologist who made a judgment regarding the presence of cognitive impairment. Next, participants were evaluated in person by an experienced neurologist or geriatrician blinded to all previously collected data; this person then used all available cognitive and clinical testing results from the current year’s evaluation to diagnose dementia and other common neurologic conditions affecting cognitive or physical function. The diagnosis of dementia followed the criteria of the joint working group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association. These require a history of cognitive decline and evidence of impairment in two or more domains of cognition, one of which must be memory for classification of Alzheimer’s disease.

**Table 1. Baseline characteristics of memory and aging participants**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Whole cohort</th>
<th>Worse quintile global motor</th>
<th>Best quintile global motor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>80.5 (±6.88)</td>
<td>83.5 (±7.34)</td>
<td>75.7 (±7.04)</td>
</tr>
<tr>
<td>Female sex</td>
<td>74.4%</td>
<td>85.1%</td>
<td>55.4%</td>
</tr>
<tr>
<td>Education (years)</td>
<td>14.5 (±3.01)</td>
<td>13.9 (±2.98)</td>
<td>15.4 (±3.33)</td>
</tr>
<tr>
<td>Mini-Mental Status Examination</td>
<td>27.9 (2.00)</td>
<td>27.1 (±2.47)</td>
<td>28.5 (±1.82)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.3 (±5.28)</td>
<td>28.1 (±6.72)</td>
<td>27.2 (±4.83)</td>
</tr>
<tr>
<td>Global motor (z-score)</td>
<td>−0.04 (±0.59)</td>
<td>−0.85 (±0.22)</td>
<td>0.80 (±0.26)</td>
</tr>
<tr>
<td>Motor performance (z-score)</td>
<td>−0.03 (±0.70)</td>
<td>−0.97 (±0.33)</td>
<td>0.80 (±0.35)</td>
</tr>
<tr>
<td>Muscle strength (z-score)</td>
<td>−0.05 (±0.71)</td>
<td>−0.66 (±0.39)</td>
<td>0.80 (±0.66)</td>
</tr>
</tbody>
</table>

* Mean (± standard deviation) unless otherwise noted.
Assessment of Physical Activity. Physical activity was assessed using questions adapted from the 1985 National Health Interview Survey. Activities included walking for exercise, gardening or yardwork, calisthenics or general exercise, bicycle riding, and swimming or water exercise. Participants were asked if they had engaged in any of those activities within the past 2 weeks and, if so, the number of occasions and average minutes per occasion. Minutes spent engaged in each activity were summed and expressed as hours of activity per week, as previously described.

Motor Function Testing. A composite measure of global motor function based on subcomponents of muscle strength and motor performance were used in the present study. Composite measures typically have metric properties that are more appropriate than individual tests for longitudinal analyses and have been used effectively in several other longitudinal studies of cognitive and motor function. Composite measures were used in this study because they yield more stable measures of motor function and increase power to identify risk factors for as well as consequences of motor decline in aging.

Appendicular muscle strength was measured using portable hand-held dynamometers (Lafayette Manual Muscle Test System, Model 01163, Lafayette, Indiana) that are reliable in older persons. The hand-held dynamometer was used to assess muscle strength in both arms (arm abduction, arm flexion, arm extension) and both lower extremities (hip flexion, knee extension, plantar flexion, and ankle dorsiflexion). Grip and pinch strength were measured bilaterally using the Jamar hydraulic hand and pinch dynamometers (Lafayette Instruments, Lafayette, Indiana). The mean score for each muscle group was converted to a z-score, using the baseline mean and standard deviation of all study participants, and the z-scores were averaged to yield a composite measure of muscle strength.

Motor performances were tested in both upper and lower extremities. In the lower extremities, we used seven performance-based tests of lower-extremity function. We asked people to walk 8 feet and turn 360° and measured the time and number of steps taken on each task. Although most individuals in our cohort initially were able to walk, during follow-up some participants were no longer able to do so. Therefore, by using a six-point scale from 0–5 and assigning 0 to those unable to walk, we were able to include all participants so as not to lose data for the longitudinal analyses. Those unable to perform a task were given a score of 0; for the remainder, scores on each distribution were divided into quintiles and scores of 5 were assigned to the quintile with the fastest time and fewest steps and scores of 1 to the quintile with the slowest times and most steps. We asked people to stand on each leg for 10 s. A score of 0 was given to those unable to perform the task. For everyone else, the total standing time for the two legs was divided into quintiles, with a score of 5 assigned to the longest times and a score of 1 to the briefest. Persons were asked to stand on their toes for 10 s, with scores of 0 to 5 assigned based on the ability to perform the task and quintile of time of the position was maintained (5 for longest, 1 for briefest). We also asked people to walk an 8-foot line in a heel-to-toe manner and counted the number of steps off line. If the foot was partially or completely off line, or even when the heel was in front and not displaced from the other foot but the rest of the foot was displaced laterally off line, these steps were counted as errors. Persons unable to attempt the task were scored 0. Steps off line were divided into quintiles for the remaining persons and a score of 5 was assigned to the least steps off line and a score of 1 to the most steps off line.

Although these seven lower-extremity performances were not particularly normally distributed, we conducted a factor analysis and two factors emerged with eigenvalues of 1 or greater; together, these factors accounted for 75% of the variance. We then conducted a factor analysis with varimax rotation, specifying two factors. Examining the highest weights, the time and steps taken to walk 8 feet and turn around loaded (median loading, 0.82; range, 0.75–0.87) on a mobility function factor, and the one leg stand, toe stand, and tandem gait variables loaded (median loading, 0.83; range, 0.78–0.85) on a balance factor. Component measures of gait and balance were then formed by converting each of the seven motor tests to a z-score using the baseline mean and standard deviation (SD) for the entire cohort, and averaging the z-scores together to form gait and balance measures.

Two tests of upper-extremity motor performance were used. The number of pegs that could be placed (Purdue pegboard) in 30 s was recorded. Two trials were recorded for each hand. The four trials were averaged to provide a Purdue pegboard score. In addition, participants tapped an electronic tapper (Western Psychological Services, Los Angeles, California) with their index finger as quickly as possible for 10 s. Two trials were performed for each hand. The four trials were averaged together to yield a tapping score. A measure of upper-extremity motor
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performance was created by converting Purdue pegboard and finger tapping scores to z-scores and then computing the average of the z-scores.

A composite measure of muscle strength was created by averaging the z-scores for arm and leg strength. A composite measure of motor performance was made by averaging the z-scores for gait, balance, and upper-extremity motor performance. A composite measure of global motor function was created by averaging all of the motor function tests together.

**Other Covariates.** Gender, race, and ethnicity were recorded at the baseline interview. Race and ethnicity questions and categories were those used by the 1990 US Census. Age in years was computed from self-reported date of birth, and date of the baseline clinical examination was that at which the strength measures were first collected. Education (reported highest grade or years of education) was obtained at the time of the baseline cognitive testing. Weight and height were measured and recorded at each visit by a trained technician blinded to previously collected data. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

In order to assess the influence of vascular risk factors and vascular disease burden on the association of motor function and mortality, we summarized cumulative vascular risk factors and vascular disease burden. For the purpose of this study, smoking history, heart attack, congestive heart failure, and claudication were rated as absent or present (0 or 1) as determined by self-report; hypertension and diabetes were rated as present if the participant reported having been diagnosed with the condition or was found to be on medication for the condition, and stroke was diagnosed based on self-report plus clinical examination, as previously described. In order to assess the influence of cumulative vascular risk factor and vascular disease burden on motor function, we computed summary scores indicating each individual’s number of vascular risk factors (i.e., the sum of hypertension, diabetes mellitus, and smoking, resulting in a score from 0–3 for each individual [mean, 1.18; SD, 0.79]) and vascular disease burden (i.e., the sum of heart attack, congestive heart failure, claudication, and stroke, resulting in a score from 0–4 for each individual [mean, 0.39; SD, 0.66]). These summary scores were used as covariates in the analyses.

Disability was assessed using two standard disability measures that capture different aspects of daily functions. We assessed six basic activities of daily living via the Katz Activities of Daily Living Scale, which included walking across a small room, bathing, dressing, eating, transferring, and toileting. A composite measure was created by summing the nonmissing items with 1 representing “can do” and 0 “cannot do” (range, 0–6). We also assessed a series of instrumental activities of daily living (IADL) with eight questions, such as household management and self-care questions, which are required for independent living.

**Statistical Analyses.** Spearman correlations were used to assess the relationship of measures of motor function with age and education, and t-tests were used to compare motor function in men and women. We used generalized estimating equation models to characterize change over time in the global measure of motor function and to test the relation of physical activity with the baseline level and rate of change in motor function. The core model with global motor function as the outcome included terms for time in years since baseline, physical activity, and the interaction of time with physical activity. The term for time indicates the expected change in motor function for a person who is not engaged in any physical activity; the term for physical activity indicates the average difference in motor function at baseline associated with a 1-h difference in physical activity; and the interaction of physical activity with time represents the effect of each additional hour of physical activity on the annual rate of change in motor function. These and all subsequent models included terms to control for the potentially confounding effects of age, sex, and education on baseline level of, and change in, motor function.

Next, we added an additional term to the core model to examine whether there was an interaction of sex with physical activity. Because persons who are disabled might be less likely to engage in physical activity, we excluded participants with nonzero Katz and IADL disability scores and repeated the core model described above. Next, since disability is a dynamic process, we treated Katz and IADL disability as time-varying covariates and included them in the core model to examine the extent to which the disability might affect the association between physical activity and change in motor function. We then repeated the core model adding different covariates (BMI for linear associations, a quadratic term for BMI (BMI×BMI) because both low and high values of BMI can be associated with adverse health consequences, vascular disease risk, and vascular disease burden]. Each covariate was added to the core model as well as a term for its interaction with time.
Finally, we repeated the core model using muscle strength and motor performance instead of global motor function as the outcomes to determine whether the association of physical activity varied with muscle strength and motor performance. Models were examined graphically and analytically and assumptions were judged to be adequately met. Programming was done in SAS (SAS Institute, Cary, North Carolina).32

RESULTS

Metric Properties of Measures of Motor Function. We first examined the metric properties of the measures of motor function. Global motor function was normally distributed (Fig. 1) and ranged from –1.9 to 1.9 (mean, 0.04; SD, 0.59), with higher scores indicating better performance. Global motor function was inversely related to age (r = –0.42, P < 0.001), positively associated with education (r = 0.19, P < 0.001), and men had higher levels of motor function than women (t[874] = –7.67, P < 0.001). Muscle strength was associated with motor performance (rho = 0.37; P < 0.001).

Physical Activity and Change in Global Motor Function. Baseline physical activity ranged from 0–35 h per week (mean, 3.0 h/wk; SD, 3.6 h; 10th percentile, 0 h/wk; 90th percentile, 7.0 h/wk). Physical activity was not related to age (rho = –0.04, P = 0.250), but was associated with education (r = 0.12, P < 0.001), and men had higher levels of physical activity (mean, 3.5; SD, 3.8) than women (mean, 2.8; SD, 3.5). [t[874] = –2.42, P = 0.02]. Physical activity was modestly associated with global motor function (rho = 0.17, P < 0.001), motor performance (rho = 0.15; P < 0.001), and muscle strength (rho = 0.13, P < 0.001).

To test the hypothesis that higher baseline levels of physical activity would be associated with a slower rate of decline in global motor function, we constructed a generalized estimating equation model to characterize the rate of change in global motor function and its relationship to physical activity. In these and all subsequent models, we also included terms to control for the potentially confounding effects of age, sex, and education. On average, global motor function declined at a rate of about 0.07 units per year (Table 2). Baseline level of physical activity was not associated with the baseline level of global motor function (Table 2). However, for each additional hour of physical activity at baseline, the average decline in global motor function was reduced by more than 5% (Table 2). Another way of describing the association of physical activity with global motor function is to compare it to the association of age and global motor function. Age for these analyses was centered at 80 years and there was a significant interaction between baseline age and motor function, such that for each year of age below 80 motor decline was reduced by about 6% (age × time: esti-

![FIGURE 1. Distribution of global motor function. This figure shows the distribution for the entire cohort of the composite measure of global motor function, which was constructed from motor performance and muscle strength.](image)

<table>
<thead>
<tr>
<th>Table 2. Association of physical activity with change in motor function*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome</strong></td>
</tr>
<tr>
<td>Global motor</td>
</tr>
<tr>
<td>Physical activity</td>
</tr>
<tr>
<td>Physical activity × Time</td>
</tr>
<tr>
<td>Motor performance</td>
</tr>
<tr>
<td>Physical activity</td>
</tr>
<tr>
<td>Physical activity × Time</td>
</tr>
<tr>
<td>Muscle strength</td>
</tr>
<tr>
<td>Physical activity</td>
</tr>
<tr>
<td>Physical activity × Time</td>
</tr>
</tbody>
</table>

*All three generalized estimating equation models included terms to control for age, sex, education and their interactions with time.
The effect of baseline physical activity on the rate of change in global motor function is illustrated in Figure 2, which shows the trajectory of motor decline for a participant with 0 h of physical activity per week (10th percentile) and the solid line shows the trajectory for a participant with 7 h of physical activity per week (90th percentile).

Because we found that there were level differences in motor function between men and women, we examined whether there was a sex difference in the association between baseline physical activity and change in motor function. We added an interaction term between sex and physical activity as well as its interaction with time. There was no significant sex difference in level of physical activity at baseline (sex × physical activity term: estimate, 0.000; SE, 0.007; P = 0.953) or sex difference in the association between baseline physical activity and change in motor function (time × sex × physical activity: estimate, 0.000, SE, 0.002; P = 0.970). This suggests that the association of physical activity with the rate of change in motor function was similar in men and women.

**Physical Activity, Other Covariates, and Motor Function.** Disability is associated with decreased motor function and might contribute to decreased physical activity. Therefore, to examine the potential influence of disability status on the above findings we excluded all participants with nonzero Katz disability scores at baseline (89 of 876; 10.2%) and repeated the core model described above. This enabled us to determine the extent to which the inclusion of persons with disability influenced the association of physical activity and change in motor function. The results were essentially unchanged (estimate, 0.005; SE, 0.001; P < 0.001). Next, we excluded all participants with nonzero IADL disability scores at baseline (437 of 876; 50%) and the results were unchanged (estimate, 0.004; SE, 0.001; P = 0.005). Since disability is a dynamic process, we then repeated the core model with all participants but added a time-varying covariate for disability to examine whether disability affected the association between baseline physical activity and motor decline. Again, the results were essentially unchanged (estimate, 0.003; SE, 0.001; P = 0.003) or IADL disability (estimate, 0.004; SE, 0.001; P < 0.001). These findings suggest that disability is unlikely to account for the observed association of physical activity and change in motor function.

Because chronic diseases may contribute to motor impairment, we added terms for vascular risk factors and vascular disease burden and their interactions with time to examine whether they affected the association of physical activity with motor function. Including a term for vascular risk factors did not change the extent to which physical activity was associated with motor function (estimate, 0.004; SE, 0.001; P < 0.001); similarly, adding a term for vascular disease burden did not change the association of physical activity with motor function (estimate, 0.003; SE, 0.001; P < 0.001).

Because muscle mass might affect motor function, we also included terms for BMI as well as BMI × BMI and their interactions with time to determine the extent to which body composition might affect the association of physical and motor function. The addition of these terms for body composition did not change the association between physical activity and motor function (estimate, 0.003; SE, 0.001; P < 0.001).

**Physical Activity and Change in Motor Performance and Muscle Strength.** The association of physical activity with motor performance and muscle strength might vary. We therefore repeated the core model described above, first replacing global motor function...
with motor performance and then with muscle strength. The association of physical activity with change in motor performance was similar to that with global motor function (Table 2). However, by contrast, there was no association between physical activity and change in muscle strength (Table 2). These results suggest that the association of physical activity with rate of change in global motor function is primarily due to its effect on motor performance.

DISCUSSION

In a cohort of more than 850 older persons without dementia, we found that a higher level of physical activity was associated with a slower rate of motor decline. Moreover, the association of physical activity with motor function persisted even after controlling for several other potentially confounding variables such as disability status, vascular risk factors and vascular disease, and body composition. Secondary analyses indicated that the association of physical activity with the rate of motor decline was due primarily to the effect of physical activity on motor performance rather than muscle strength.

Older persons often experience and complain of progressive loss of motor function.2,36 Motor decline is associated with disability, cognitive decline, institutionalization, and mortality.1,14,15 Identifying risk factors or behaviors that can reduce or modify motor decline in aging may have important public health benefits. Exercise intervention studies have demonstrated the benefits of physical activity in improving balance and decreasing the risk of falls, but these studies have relatively short durations, usually months, of follow-up.22 One recent study in older women showed an association between level of physical activity and gait speed more than 10 years later,8 and a second study of both men and women showed that a higher level of physical activity was associated with better mobility 3 years later.38 The present study extends prior studies by using repeated measures of motor function that tend to minimize the effects of the baseline level of function. This study shows that physical activity is associated with better motor function in aging because a higher level of activity is associated with a slower rate of motor decline. In addition, despite level differences in motor function between men and women, physical activity affects motor decline similarly in men and women. The benefit of physical activity in the present study was not only observed in the lower extremities but was more generalized, since the composite measure of motor function was derived from multiple strength and motor performance measures in both the upper and lower extremities. These findings suggest that, although the causes of age-related motor decline are poorly understood, physical activity is a modifiable risk factor that may slow the course of decline in motor function.

The association of physical activity with motor decline in this study was due mostly to the effect of physical activity on the rate of decline in motor performance rather than muscle strength. Importantly, a large number of cortical and subcortical structures are necessary for the planning and execution of motor performances,16 whereas muscle strength may predominantly reflect motor unit and muscle function.10,11,18,30 Recent work has shown that the beneficial effect of physical activity on mobility is not entirely due to its effect on the lower extremity muscles, suggesting that physical activity may exert its benefit through its effect on central motor structures.59 In addition, although there is an extensive literature documenting the beneficial effect of physical activity for muscle function as well as cardiovascular fitness,27,41 recent evidence suggests that physical activity may also have direct effects on spinal motor neurons.3,4,13,17,19,34,35 Current work also suggests that physical activity is associated with a host of positive health benefits that extend throughout the central nervous system, including maintaining neuronal plasticity and increasing neurogenesis.6,12,21,37 There is additional evidence that physical activity may also stimulate the growth and development of brain cells and protect against ischemic and neurotoxic damage.12,21 Further work is needed to determine the precise mechanisms and extent to which physical activity affects different aspects of movement in older persons.

Our study has some limitations. First, motor function testing, particularly measures of muscle strength, are vulnerable to practice effects in the first years of follow-up. Given that the duration of follow-up was relatively short in this study, practice effects may account for the limited rate of change in muscle strength. If this is the case, then we may have underestimated the actual contribution of physical activity to change in strength. In addition, because persons who were demented at baseline or died before the first follow-up evaluation were not eligible for analyses, these results may underestimate the magnitude of the association between physical activity with motor function. In this study, vascular risk factors and disease burden were based primarily on participants’ self-report and objective measures would lend more precision to the extent to which these disorders may affect motor function. Similarly, physical activity measures were based on self-
reported activity. Quantitative measures of physical activity such as measures of intensity and energy expenditure would provide more accurate information about the frequency and duration of physical activity.

Despite these limitations, several factors increase confidence in the findings from this study. Perhaps most important, motor function was evaluated as part of a uniform clinical evaluation and incorporated many widely accepted and reliable strength and motor performance measures; strength testing was done in all four extremities, and motor performances were tested in both the arms and legs. The aggregation of multiple measures of motor function into composite measures of muscle strength and motor performance is likely to yield a more stable measure of motor function and increase statistical power to identify risk factors as well as the adverse health consequences of motor decline in aging. In addition, persons with dementia were excluded and a relatively large number of older persons representative of the general population were studied, so that there was adequate statistical power to identify the associations of interest while controlling for potentially confounding demographic variables.

This work was supported by National Institute on Aging grants R01AG17917 and R01AG024480, the Illinois Department of Public Health, and the Robert C. Borwell Endowment Fund. We thank all the participants in the Rush Memory and Aging Project. We also thank Traci Colvin, Tracy Hagman, and Tracey Nowakowski for project coordination; Barbara Eubeler, Mary Funnel, Karen Lowe Graham, and Pamela Smith for participant recruitment; George Dombrowski and Greg Klein for data management; Zhaotai Cui for statistical programming; and the staff of the Rush Alzheimer’s Disease Center and Rush Institute for Healthy Aging.

REFERENCES

ABSTRACT: Stretching is widely used in rehabilitation and sports activities to improve joint range-of-motion and flexibility in humans, but the effect of stretching on the gene expression of skeletal muscle is poorly understood. We evaluated the effect of short bouts of passive stretching of rat soleus muscle on myo-D, myostatin, and atrogin-1 gene expressions. Six groups of animals were submitted to a single session of stretching (10 stretches of 1 minute with 30 seconds of rest between them, performed manually) and were evaluated immediately (I), and 8, 24, 48, 72, and 168 hours after the session. To evaluate the effect of repetitive sessions of stretching on the soleus muscle over 1 week, three groups of animals received a single session per day of stretching and the muscle was evaluated immediately after 2, 3, and 7 sessions. The mRNA levels of myo-D, myostatin, and atrogin-1 were determined by real-time polymerase chain reaction. A single session of stretching increased the mRNA levels of myo-D (after 24 h), myostatin (I, and 168 h later), and atrogin-1 (after 48 h). Repeated daily session of stretching over 1 week increased myostatin (after 7 sessions) and atrogin-1 expression (after 2, 3, and 7 sessions). Thus, short bouts of passive stretching are able to increase the gene expression of factors associated with muscle growth (myo-D), negative regulation of muscle mass (myostatin), and atrophy (atrogin-1), indicating muscle remodeling through different pathways.

SHORT BOUTS OF STRETCHING INCREASE MYO-D, MYOSTATIN AND ATROGIN-1 IN RAT SOLEUS MUSCLE

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Skeletal muscle stretching prevents muscle-fiber atrophy, connective tissue proliferation, and loss of serial sarcomeres in immobilized muscles. As muscle atrophy is associated with protein degradation, stretching is a powerful mechanical signal of muscle protein synthesis and growth. Although the mechanism by which nuclei increase transcription of specific skeletal muscle messenger ribonucleic acid (mRNA) in response to a hypertrophic stimulus is unknown, myogenic regulatory factors (MRFs) may be involved in this process.

MRFs are a family of skeletal muscle-specific transcription factors that control the expression of several muscle genes, such as desmin, troponin I, and myosin light chain. MRFs are composed of four members: myo-D, myf-5, myogenin, and MRF-4. MRFs are upregulated in skeletal muscle hypertrophy induced by stretching. One of the foci of the present study was to investigate myo-D (myogenic differentiation 1) gene expression in skeletal muscle submitted to stretching, due to the role of myo-D in muscle hypertrophic response, and also because MRFs respond to passive stretching according to time, age, and muscle type.
Although muscle stretching is a hypertrophic stimulus, some reports also described a decrease in the cross-sectional area of rat soleus muscle submitted to stretching. Thus, in the present study, the effect of stretching on the genes related to the regulation of muscle mass (myostatin) and atrophy (atrogin-1) was also investigated.

Myostatin (growth and differentiation factor-8; GDF-8) was identified by McPherron et al. in mice as a transforming growth factor-beta (TGF-β) family member synthesized by skeletal muscle, which circulates in the blood and acts in a concentration-dependent manner as a negative regulator of muscle growth. Myostatin levels increase during periods of muscle inactivity, whereas myostatin expression is reduced on muscle reloading. Moreover, blockage and subsequent inhibition of serum myostatin increased total body mass, muscle mass, muscle size, and absolute muscle strength. Although it is reasonable to assume that physical activity decreases the expression of myostatin, two recent reports described an increase in the transcript levels of myostatin in muscles submitted to eccentric training in both rats and humans. In the present study, myostatin was investigated in an attempt to determine whether its expression is affected by short bouts of passive stretching.

Atrogin-1 is a gene strongly activated in atrophying muscles regardless of etiology. Atrogin-1, also known as MAFbx (muscle atrophy F-box), is an F-box protein that links the protein substrate to be ubiquitinated and degraded with the rest of the E3 and ubiquitination machinery. Daily 30-minute sessions of passive stretching increase the expression of the atrogin-1 gene. Thus, atrogin-1 can be considered as a good candidate to study possible muscle atrophy associated with passive stretching.

Models to investigate the effect of stretching on skeletal muscle plasticity frequently use chronic passive stretching, applying immobilization using a plaster cast or adhesive tape. Nevertheless, to our knowledge, only three studies have reported the effect of short periods of stretching on the gene expression of skeletal muscle, and all investigated the rat soleus muscle. The first study showed an increase in the expression of myogenin after repetitive stretching (15 times per minute for 4 hours). The second study reported an increase in myogenin gene expression in muscle submitted to repetitive stretching for 60 minutes. The third study reported an increase in myo-D and atrogin-1 gene expressions after 30-minute sessions of passive stretching.

Although these studies have contributed to knowledge about the effect of stretch on gene expression of skeletal muscle, most used long periods of muscle stretching, not normally used in humans during rehabilitation or sports activities. By contrast, short bouts of muscle stretching (30 seconds or 1 minute) have been recommended to treat shortened human muscles because of their effectiveness in improving joint range-of-motion and flexibility. Although sessions of short stretching periods are recommended, safe, and effective for humans, the effects of this procedure on skeletal muscle have not been evaluated in detail.

Thus, the hypothesis of this study was that genes related to muscle remodeling are altered by short bouts of stretching, as recommended for humans. For this, we evaluated the expressions of three genes in the rat soleus muscle: myo-D, related to muscle growth; myostatin, known as a negative regulator of muscle mass; and atrogin-1, a gene involved in muscle atrophy.

MATERIALS AND METHODS

Animal Care and Experimental Groups. Fifty male, 3-month-old Wistar rats were used (weight: 373 ± 32 g) for the study. They were housed in plastic cages in a room with controlled environmental conditions and had free access to water and standard food. The experimental procedures were approved by the ethics committee of our university and conducted in accordance with the Guide for Care and Use of Laboratory Animals. The animals were randomly divided into 10 groups of 5 animals each. The rats were anesthetized by intraperitoneal injections of xylazine (12 mg.kg⁻¹) and ketamine (95 mg.kg⁻¹) for the stretching procedure and muscle dissection. They were then euthanized by an overdose of the anesthetic.

To stretch the left soleus muscles, the left ankle was held manually in full dorsiflexion for 1 min, as described by Ikeda et al. A single session of passive stretching consisted of 10 bouts of stretching, each maintained for 1 min, with rest for 30 s between bouts. Six groups of animals received only a single session of stretching and were evaluated immediately after (I), and 8 h, 24 h, 48 h, 72 h, and 168 h later. To evaluate the effect of repetitive sessions of stretching on the soleus muscle over the course of 1 week, three groups of animals received a single daily session of stretching and the muscle was evaluated immediately after 2, 3, and 7 sessions; that is, the group “2 sessions” was daily stretched for 2 days only, the group “3 sessions” for 3 days only, and the group “7 sessions” for 7 days only. One group of animals...
was not submitted to either procedure and the soleus was used as a control.

The soleus muscle was chosen for this study because it crosses only the ankle joint and histological cross-sections taken from the middle belly contain all muscle fibers, avoiding sampling problems. Also, this muscle has been widely used in previous studies of stretch on skeletal muscle.14,18,19,45 The left soleus muscle was dissected, excised, and weighed. It was then cut into four equal lengths between the proximal and distal ends, using a caliper. The distal ends of the muscle were immediately frozen in liquid nitrogen and stored at −80°C for the extraction of total RNA. As there are conflicting reports in the literature regarding the distribution of MRFs along stretched muscle fibers,11,24,51 we used only the ends of the soleus muscle for the evaluation of gene expression.

Muscle Morphology. To evaluate the possible presence of signs of muscle injury induced by the stretching sessions, histological cross-sections of the soleus muscles were analyzed. For this, the medial parts of the middle belly muscle were frozen in isopentane pre-cooled in liquid nitrogen and stored in a freezer (−80°C). Serial cross-sections (10 µm thick) were then obtained from the frozen soleus muscle using a microtome cryostat (HE 505; Microm, Jena, Germany) maintained at −20°C and alternate serial cross-sections of the muscles stained with 1% toluidine blue or submitted to acid phosphatase.5 Toluidine blue staining was used to evaluate the morphological pattern of the muscle fibers and the presence of muscle-fiber injury, because it permits the identification of the myonuclei, areas of myonecrosis, and the basophilic regions of the muscle fibers, as previously described.44 Acid phosphatase was used to identify signs of necrosis. Normal muscles fibers do not show a positive acid phosphatase reaction that indicates a high concentration of lysosomes, which is considered as proof of tissue necrosis and phagocytosis.

RNA Isolation and Analysis. RNA was isolated from one frozen fragment from the distal ends of each muscle using 1 ml of Trizol reagent (Invitrogen, Carlsbad, California), according to the manufacturer’s instructions. The extracted RNA was dissolved in Tris-HCl and ethylene-diamine tetraacetic acid (pH 7.6) and quantified spectrophotometrically. The purity was assessed by determining the ratio of the absorbance at 260 nm and 280 nm. All samples had 260/280-nm ratios above 2.0. The integrity of the RNA was confirmed by inspection of ethidium bro-
RESULTS

No difference was found in the weight of the soleus muscle among the groups evaluated. The soleus muscles in all the groups of animals exhibited normal muscle fibers. No signs of injury were found in the soleus muscles submitted to stretching.

**Gene Expression.** After a single stretching session, there was an increase in myo-D gene expression of the soleus muscle after 24 hours (1.69 $\pm$ 0.4 fold, $P < 0.05$; Fig. 1A), compared to controls. However, the myo-D gene expression was not altered after daily stretching sessions (Fig. 1B).

An increase in myostatin gene expression was found in the soleus muscle immediately after a single stretching session (1.63 $\pm$ 0.2 fold, $P < 0.0005$) and 168 hours later (1.34 $\pm$ 0.04 fold, $P = 0.01$), as compared to the control group (Fig. 2A). Myostatin gene expression also increased after 7 daily stretching sessions (1.60 $\pm$ 0.27 fold, $P = 0.01$), compared to controls (Fig. 2B).

The soleus muscle showed an increase in atrogin-1 gene expression 48 hours after a single stretching session (3.08 $\pm$ 0.48 fold, $P < 0.0001$; Fig. 3A), compared to the control group. Atrogin-1 gene expression also increased after 2 (3.77 $\pm$ 0.5 fold, $P < 0.005$), 3 (4.26 $\pm$ 0.6 fold, $P < 0.0005$), and 7 (3.27 $\pm$ 0.5 fold, $P < 0.005$) daily stretching sessions, compared to controls (Fig. 3B).

DISCUSSION

A single session of 10 passive 1-minute stretches of rat soleus muscle increased the mRNA levels of myo-D, myostatin, and atrogin-1. However, daily stretching sessions only increased myostatin and atrogin-1 gene expression. Short bouts of stretching are able to stimulate skeletal muscle remodeling through different pathways. Our study has provided new information about the expression of these genes in skeletal muscle submitted to short bouts of stretching, similar to that used in humans during...
rehabilitation and in sports activities to maintain or recover the joint range of motion and flexibility.\textsuperscript{3,4}

Skeletal muscles of quails\textsuperscript{31,32} and rats,\textsuperscript{24,51} immobilized in the stretched position, exhibit an increase in myo-D gene expression, and it was recently reported that a single 30-minute session of passive stretching also increased myo-D gene expression of rat soleus muscle.\textsuperscript{19} However, in all these studies, the muscles were maintained immobilized in the stretched position, which is not normally recommended for human skeletal muscle during rehabilitation and sports activities. The results of our study showed that short bouts of passive stretching, a procedure commonly used in humans, increased myo-D gene expression in rat muscle.

Studies have shown conflicting results with regard to myo-D gene expression. Previous reports using chicken muscles as a model found no change in myo-D mRNA levels after 3, 6, 14, and 21 days of immobilization in the stretched position.\textsuperscript{11,12} By contrast, Eppley et al.\textsuperscript{15} observed high expression of qmfi, an avian homolog of myo-D, after 3–16 hours in muscles submitted to a model of stretch-induced injury. Zador et al.\textsuperscript{51} found an increase in myo-D expression of rat soleus muscle maintained in an extended position for 3 days. Hill and Goldspink\textsuperscript{24} reported an increase in myo-D gene expression of rat tibialis anterior muscle, after 1 day of immobilization in the stretched position, associated with 1 hour of electrical stimulation. An increase in myo-D transcription of rat tibialis anterior muscle was also found 3 hours after a single bout of 30 eccentric contractions applied to the muscle maintained in the stretched position.\textsuperscript{36}

Considering the aforementioned studies, the discrepancies in myo-D gene expression can be attributed to differences in animal species, muscles, and stretching protocols. One possible explanation for the increase in myo-D mRNA level may be a surge of myo-D in proliferating satellite cells soon after stretching. When activated, satellite cells can proliferate, differentiate, and fuse with existing muscle fibers or with each other.\textsuperscript{42} Satellite-cell proliferation typically begins 24–48 hours after regeneration and, during subsequent events, MRFs are expressed in these cells.\textsuperscript{21} Mononuclear cells with myo-D peak expression were noted even after 24 hours.\textsuperscript{21} However, previous studies showed that the myo-D mRNA level was elevated even in stretched-overloaded muscles irradiated to eliminate satellite-cell proliferation, providing evidence that myo-D activation is not dependent only on satellite-cell proliferation.\textsuperscript{31}

Insulin-like growth factor-1 (IGF-1) can induce skeletal muscle hypertrophy by activating the phosphatidylinositol 3-kinase (PI3K)–serine/threonine kinase (Akt) pathway.\textsuperscript{16} Interaction between Rho, a guanosine triphosphate (GTP)–binding protein with GTPase activities, and the serum response factor (SRF), a DNA-binding protein, is required for the regulatory pathway that controls myo-D gene expression, and Rho/SRF activities are dependent on IGF factors.\textsuperscript{9,10,23} Thus, it is tempting to speculate that if IGF is related to hypertrophy and myo-D activation depends on Rho/SRF activation, the data from the present work could mean that a single session of short stretching bouts can produce a hypertrophic signal, as observed by the increase in the myo-D mRNA level.

However, it was recently reported that the induction of the IGF-1/PI3K/Akt pathway prevents the induction of requisite atrophy mediators such as atrogin-1.\textsuperscript{14} Also, Langley et al.\textsuperscript{29} demonstrated that myo-D synthesis could be inhibited by myostatin, in a myoblast culture, suggesting a negative regulatory
role of myostatin on myo-D. Interestingly, atrogin-1/MAFbx can also modulate myo-D expression. It was demonstrated that upregulation of atrogin-1/MAFbx in proliferating myoblasts antagonizes differentiation, inducing myo-D degradation and preventing muscle-specific gene activation. The absence of changes in myo-D expression after daily stretching sessions could be associated with the increased expression of atrogin-1. Also, in the present study, atrogin-1 and myo-D were never concomitantly up-regulated in stretched muscles. Therefore, the number of stretching stimuli contributed to regulate the expression of both myo-D and atrogin-1 genes.

Regarding myostatin, the results concerning its expression in skeletal muscle loading are controversial. Studies in animals have revealed reduced myostatin expression in response to reloading after atrophy conditions. However, eccentric exercise induced an upregulation in myostatin mRNA expression, whereas concentric training reduced it.

In humans, a reduction in myostatin expression occurred with heavy-resistance strength training. However, no change in myostatin expression was found following 2 weeks of immobilization, although it decreased 24 hours after cast removal and the start of exercise rehabilitation. It has been observed that myostatin levels increase very fast (after 30 min) after a bout of eccentric exercise in dorsiflexor muscles of rats, and another study also identified an increase in myostatin mRNA level in response to heavy-resistance training, which appears to have no effect on muscle strength and mass in humans. Together these findings also suggest that the level of stress in the muscle fibers could be a determinant factor in the responsiveness of the myostatin gene.

The one recent report addressing the effect of passive stretching on myostatin gene expression showed an absence of change in the expression of this gene in rat soleus muscles submitted to 30-minute stretching. In contrast, our results identified a significant increase in myostatin gene expression in rat soleus muscle after different periods (immediately and after 168 h) following a single stretching session, as well as after 7 daily stretching sessions. Although the level of muscle stress and tension were not analyzed, a possible explanation for the differences between these two studies with respect to myostatin expression is the level of stress induced in the soleus muscle.

The different peaks of myostatin expression identified after a single session of stretching are intriguing (Fig. 2). Another report also showed different peaks of gene expression at different periods of time after stimulation. The investigators suggested that these effects depend on different molecular mechanisms. They related the first peak of gene expression to an increase in rate of transcription, and the later increase to mRNA stability.

The effect of muscle stretching on atrogin-1 gene expression has not been studied previously, except in a recent report that found an increase in atrogin-1 mRNA levels in the rat soleus muscle after daily 30-minute stretching sessions. Our results showed that a single stretching session, as well as daily stretching sessions, increased atrogin-1 mRNA levels (Fig. 3), which indicates that short bouts of passive stretching activate the proteasome pathway in rat soleus muscle. This result is in accordance with previous reports that found a decrease in muscle-fiber cross-sectional area after stretching, in rat soleus muscle, suggesting involvement of cellular degradation pathways. Stretching probably causes muscle-fiber remodeling and interferes with both hypertrophy and atrophy pathways. Also, it could act differently on radial and longitudinal muscle growth. For example, muscle stretched once per week shows a decrease in cross-sectional area without modification in the serial sarcomeres. Shortened muscles may respond differently to stretching than muscles with normal length. However, further studies are necessary to test this hypothesis. The absence of muscle-fiber injury in the muscles submitted to stretching sessions, associated with the increased gene expression of myo-D, myostatin, and atrogin-1, suggests that the muscle remodeling induced by short stretching bouts is not associated with muscle-fiber injury.

Changes in mRNA levels do not necessarily mean changes in protein rates. Studies that evaluate both gene expression and protein rates of myo-D, myostatin, and atrogin-1 are necessary and could contribute to a better understanding of the effects of stretching on skeletal muscle remodeling.

In conclusion, short bouts of passive stretch, as recommended to improve joint range-of-motion and flexibility in humans, increase the gene expression of factors associated with muscle growth (myo-D), negative regulation of muscle mass (myostatin), and atrophy (atrogin-1), indicating muscle remodeling through different pathways.

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ABSTRACT: The effect of age on mechanomyography (MMG) has not been examined for electrically evoked contractions. Similar to torque, we expected that postactivation potentiation of the MMG would differ between young and old subjects. Additionally, under voluntary conditions, we compared normalized MMG and electromyographic (EMG) signals in relation to torque, and expected that MMG, unlike EMG, would be affected by age. In 10 young and 10 old men, electrical stimulation was delivered before and after a 10-s maximal voluntary contraction (MVC) to assess potentiation of contractile (twitch torque; Pt), electrical (M-wave amplitude), and mechanical (MMG amplitude) properties of the dorsiflexors. Subsequently, subjects performed voluntary contractions at 20%, 40%, 60%, 80%, and 100% MVC for calculation of normalized MMG–torque and EMG–torque relationships. Following the MVC, Pt and evoked MMG were larger than at rest in both groups, but M-wave amplitude was unchanged. Twitch potentiation was greater in young than old, whereas evoked MMG was unaffected by age. Under voluntary conditions, values for MMG and EMG were similar between groups, except for greater MMG at MVC in young men. The shape of MMG and EMG relationships to torque was similar only in young men. Using the aging model, our results indicate that potentiation of the mechanical components (MMG) differ from those of twitch torque. Furthermore, the comparison of normalized voluntary MMG with age provides additional support for the concept of age-related motor unit remodeling.


MECHANOMYOGRAPHIC AND ELECTROMYOGRAPHIC RESPONSES TO STIMULATED AND VOLUNTARY CONTRACTIONS IN THE DORSIFLEXORS OF YOUNG AND OLD MEN

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The responses of muscle to either voluntary or electrical excitation can be assessed by recording contractile properties across the joint (force or torque), electrical events associated with excitation (electromyography, EMG) or mechanical properties of the muscle during contractile activation (mechanomyography, MMG). Most often, measures of contractile properties and EMG have been combined, but recently the MMG technique has been applied as an additional measure to assess contractile function.1,4,17,30,31,39 The MMG signal recorded on the skin overlying the active muscle is a compound mechanical signal that records changes in muscle contractile movements that are not influenced by series elastic and tendon compliances.5 MMG may therefore provide a more direct estimate of muscle force generation of a specific muscle than joint force or torque.39 Furthermore, unlike the EMG signal, which is influenced mainly by a combination of motor unit (MU) recruitment and MU rate coding, the MMG is influenced by these factors and the subsequent mechanical fusion of MU contractile properties. Thus, MMG can provide additional information on factors affecting force generation that complement the information obtained from EMG. For example, during vol-

Abbreviations: EMG, electromyography; MMG, mechanomyography; MU, motor unit; MVC, maximal voluntary contraction; Pt, peak twitch torque; RMS, root-mean-square; TA, tibialis anterior
Key words: aging; mechanomyogram; tibialis anterior muscle; twitch potentiation
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untary contractions EMG and MMG are each positively related to the neural drive to the muscle, but with fatigue their responses may be dissociated. \[32\] In response to electrical stimulation, recording of the compound muscle action potential (M-wave) is used to assess electrical transmission, whereas MMG reflects the associated mechanical response of the contractile apparatus and structural properties of the muscle. Therefore, when combined with contractile properties, recordings of EMG and MMG under both voluntary and electrically stimulated contractions should provide a comprehensive understanding of factors related to changes in muscle torque output.

With advanced age, muscle contractile function and structure are substantially modified, with loss of muscle mass and strength, slowing of contractile properties,\[33,36\] decrease in tendon stiffness,\[37\] alterations in excitation–contraction coupling,\[34\] and diminished M-wave responses.\[37\] An age-related loss of MUs with compensatory MU remodeling may be one mechanism responsible for many of these changes, and indeed studies have reported that certain muscles from aged people have fewer and larger MUs than those of younger subjects.\[30,31,35\]

There are only three studies comparing MMG in young and old adults, and in each case MMG responses were assessed only during voluntary contractions from muscles affecting elbow movements.\[1,13,21\] In these studies, the absolute MMG signal recorded from voluntary contractions of varying intensities was either unchanged by age\[1,21\] or greater in the young than old adults.\[13,21\] Differences in the muscle studied, the testing protocol, and the MMG recording device may account for the variable results from these few studies. Surface EMG recordings can be affected by many variables, including skin and subcutaneous fat thickness\[16,23\] and, thus, for proper comparisons, the surface EMG signal is usually normalized and compared in relative values. Similar factors are known to affect the MMG recording\[1,4\] and, for meaningful comparisons among individuals or between groups, absolute values may be misleading. In the previous studies on MMG and aging cited above, absolute rather than relative MMG values were used to compare results between groups.

To our knowledge, there are no studies that have assessed the effect of age on MMG properties recorded during electrically evoked muscle excitation. Recently, we conducted a study in young men on the effects of stimulated twitch potentiation on MMG, and reported that evoked MMG amplitude, like twitch torque, was significantly greater than in the resting state after a 10-s maximum voluntary contrac-

**Materials and Methods**

**Subjects.** Ten young men, age 21–33 years (27.1 ± 3.8 years) and 10 old men, age 75–83 years (79.0 ± 2.5 years) volunteered for this study. Young subjects were recruited from the university environment, whereas old subjects were recruited from a local exercise program designed to maintain cardiovascular fitness, flexibility, and muscular endurance. All subjects were healthy, with no evidence of neuromuscular disease, and were considered to be moderately active for their respective age groups. The mean height and weight of the young group was 174.2 ± 7.7 cm and 78.7 ± 7.8 kg; corresponding values for the old group were 171.4 ± 5.7 cm and 79.2 ± 10.2 kg, respectively. The study was conducted in accordance with the guidelines for experimentation of the ethics review board of the University of Western Ontario, and informed written consent was obtained from all subjects. Data were collected during a single visit to the laboratory.

**Experimental Arrangement.** Subjects were seated in a custom-built isometric dynamometer with their right ankle positioned at 20° of plantar flexion to maximize voluntary torque and minimize antagonist muscle torque under evoked conditions\[35\] and an angle of 90° at both the hip and knee joints. A
C-clamp pressing down on the distal aspect of the right thigh minimized hip flexion during the dorsiflexion contractions. Velcro straps across the toes and the dorsum of the foot secured the limb to the dynamometer footplate.

The MMG signals were detected by a uniaxial accelerometer which was 9-mm square with a thickness of 4.5 mm and a mass of 0.75 g (MP110-10-101; MEDiSENS, Tokyo, Japan). The accelerometer was secured with double-sided adhesive tape over the portion of the tibialis anterior (TA) muscle belly that yielded the shortest rise-time of the initial positive wave of the evoked MMG signal. This site was ~7 cm distal to the tibial tuberosity and 2 cm lateral to the anterior border of the tibia.

Global dorsiflexor EMG was recorded with a monopolar arrangement using self-adhering electrocardiogram electrodes (1.5 × 1 cm; Kendall-LTP, Chicopee, Massachusetts). The active electrode was positioned over the TA muscle belly just proximal to the accelerometer, whereas the reference electrode was positioned over the distal tendon of the TA. A ground electrode was placed on the patella. The signal-to-noise ratio was high using this arrangement (Figs. 1, 2).

**Experimental Procedures.** Data collection began with the determination of the maximum M-wave amplitude with stimulation (DS7AH; Digitimer, Welwyn Garden City, United Kingdom) delivered over the common peroneal nerve, near the fibular head. Electrical stimulation was delivered at a pulse width of 50 μs at 400 V, and with a current intensity of 70–150 mA. The peak-to-peak amplitude of the M-wave was monitored as the current intensity was increased incrementally. When the M-wave amplitude reached a plateau, the current was increased a further 15% to ensure that stimulation was supramaximal. After a brief period of rest (30–60 s), a supramaximal twitch was delivered to assess Pt and evoked MMG amplitude in the resting (unpotentiated) state. Two seconds after the supramaximal twitch, an isometric MVC of the dorsiflexors was performed and sustained for 10 s, during which time subjects received visual torque feedback via an oscilloscope and strong verbal encouragement. Central activation was assessed during the MVC by use of the interpolated twitch technique. The torque amplitudes of supramaximal twitches (Ts) elicited during the MVC (at 2.5, 5, and 7.5 s) were compared to a resting twitch (Tr) delivered after the MVC (2 s) to quantify central activation using the formula: percent activation = \[1 - \frac{(T_s/T_r)}{100}\]. To assess postactivation potentiation, evoked twitches were delivered at several times after the MVC (2, 15, 30, and 45 s, and 1, 2, 3, and 5 min).

After the evoked twitch at 5 min, subjects performed two additional MVCs (sustained for 3 s), separated by 2 min. The purpose of these MVCs was to allow subjects more than one opportunity to produce their maximal torque before the calculation of submaximal loads for the EMG/torque and MMG/torque relationships. Rest for 2 min was provided between the final MVC and first submaximal load. During this time, a target line was set on the oscilloscope at the level of the first load. Subjects were asked to match this target torque as closely as possible during a 4-s contraction. The submaximal loads employed were 20%, 40%, 60%, and 80% MVC, performed in randomized order. One contraction was performed at each load and rest for 1 min was
provided between each contraction; during this time, the operator repositioned the target line of the oscilloscope to the next contraction intensity. A second submaximal contraction was performed when the subject under- or overshot the target torque by $\pm 5\%$, or did not maintain a steady contraction for at least half of the contraction time.

Data Reduction and Statistics. Sensitivity of the accelerometer was 500 mV/g ($g = 9.8$ m/s$^2$). The MMG signal was amplified ($\times 20$ and $\times 1$ gain for voluntary and stimulated MMG channels, respectively) and filtered by an AC amplifier (MP110; ME-DiSENS) with a bandwidth of 1 Hz ($-30\text{dB/oct}$) to 1 kHz ($-70\text{dB/oct}$). Torque, MMG, and EMG data were sampled on-line using Spike2 (v. 4.13; Cambridge Electronic Design, Cambridge, United Kingdom) software. Using a 12-bit A/D converter (model 1401 Plus; Cambridge Electronic Design), the torque data were sampled at 500 Hz and the MMG sampled at 2,500 Hz. The EMG was wideband filtered from 10 Hz to 1 kHz, and sampled at 2,500 Hz. During off-line analysis, the voluntary MMG signal was filtered from 5 Hz to 250 Hz to reflect the lower-frequency content of the voluntary signal compared with the evoked MMG signal. M-wave amplitude was measured from peak to peak, whereas MMG amplitude of the evoked twitch was measured from baseline to initial positive peak (Fig. 1). Root-mean-square (RMS) values of the voluntary EMG and MMG signals were calculated over a 1-s interval when torque reached the target level (Fig. 2). For each subject, the RMS surface EMG during the voluntary contractions was normalized to the maximum M-wave amplitude, and the RMS of the surface MMG during voluntary contractions was normalized to the amplitude of the evoked MMG at the peak twitch torque. All 20 subjects were used in the analysis of each measure with the exception of the voluntary EMG/torque and MMG/torque relationships, for which one old subject was excluded due to a technical problem during that portion of the experiment.

Data are reported in the text and figures as means $\pm$ standard error. A two-way repeated-measures analysis of variance was used for statistical analysis of normalized Pt and evoked MMG amplitude (age by time), as well as the normalized voluntary EMG/torque and MMG/torque relationships (age by torque). When a significant main effect or interaction was found, Dunnet and Tukey’s post-hoc tests were performed to indicate where significant differences existed for age, time, and torque comparisons. Maximum voluntary torque, as well as peak torque, contraction duration, MMG amplitude, and M-wave amplitude of the resting twitch were analyzed using an unpaired $t$-test. For all statistical analyses, the level of significance was set at $P < 0.05$.

RESULTS

Maximum torque production and resting contractile properties are reported in Table 1. The Pt and MMG amplitude were not significantly different between young and old men. However, the MVC torque and M-wave amplitude of the young men were significantly greater (21% and 26%, respectively) than that of the old men, whereas twitch contraction duration was significantly shorter (11%) in the young than old men. Central activation, as determined by the twitch

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<th>Table 1. Maximum voluntary torque and resting contractile properties of the dorsiflexors in young and old men.</th>
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<td>Maximum voluntary contraction (Nm)</td>
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Values are means $\pm$ SE.

*Significant ($P < 0.05$) different between young and old men.

Twitch contraction duration : time to peak torque + half relaxation time.
interpolation method, was complete (i.e., 100%) during all maximum efforts for both young and old men.

Figure 3A shows the relative change in Pt following the 10-s MVC. The Pt was significantly increased (potentiated) from rest for at least 5 min in both groups, although the magnitude of increase was greater in the young than old men at 2 s (122%) through to 45 s (34%). The evoked MMG amplitude (Fig. 3B) was significantly higher than the resting value for up to 2 min in both young and old men but, unlike Pt, the degree of potentiation of the evoked MMG amplitude was not different between the two age groups. Potentiation of the electrical signal did not occur following the 10-s MVC in either age group, i.e., the M-wave amplitude was unchanged from rest at all times in both young and old men.

Figure 4A and B are plots of the normalized EMG–torque and the MMG–torque relationships, respectively. In both groups, RMS of the EMG signal increased with increasing contraction intensity (Fig. 4A). There was neither an age effect nor an interaction for the normalized EMG–torque relationship. The RMS of the MMG signal in the young men also increased with increasing contraction intensity; however, in the old men there was a more modest increase followed by a small decrease between 80% and 100% MVC (Fig. 4B). As a result of the differently shaped curves, there was an age by torque interaction for the normalized MMG–torque relationship such that young men had significantly greater MMG at MVC than old men.

DISCUSSION

We found, in the human dorsiflexors, that without a change in M-wave amplitude, the evoked peak twitch torque (Pt) and MMG amplitude were significantly higher following a 10-s MVC than the resting values in both young and old men. However, the potentiation of Pt was significantly higher in young than old men, whereas the evoked MMG amplitude potenti-
ation was unaffected by age. During voluntary contractions, progressively higher percentages of maximum torque corresponded to equal, curvilinear increases of normalized surface EMG for both age groups. In contrast, the normalized voluntary MMG in both old adults, although coupling mechanisms are responsible for the age-related differences in excitation–contraction coupling mechanisms. The MMG signal returned to baseline by 3 min. Of minor note, subjects experienced a greater degree of potentiation than their counterparts in the earlier two studies, an effect most likely due to our use of a longer-duration potentiation contraction.

Similar to Pt, the evoked MMG amplitude was potentiated following the 10-s MVC in both young and old men; however, unlike Pt, potentiation of the evoked MMG was not affected by age. Furthermore, Pt was significantly potentiated for >5 min, whereas the MMG signal returned to baseline by 3 min. These discrepancies between Pt and evoked MMG amplitude indicate that MMG recorded from a single muscle is distinct from joint torque recorded for a muscle group. Recent studies have suggested that age-related differences in excitation–contraction coupling mechanisms are responsible for the reduced potentiation of Pt in old adults, although other factors related to type II fiber atrophy and tendon stiffness may be involved.

In a previous study in the plantar flexors of young subjects, we suggested that, like Pt, the increased evoked MMG amplitude caused by potentiation may be explained by facilitation of the excitation–contraction coupling mechanisms. The addition in this study of a group of aged subjects with altered contractile characteristics has provided an opportunity to further understand the interplay of various factors on the MMG signal. Indeed, a possible reason for the absence of greater potentiation of MMG amplitude in our young than old subjects may be related to differences in active muscle stiffness. That is, more intense contractions result in an increase in active muscle stiffness due to greater numbers of cross-bridge attachments and increased stiffness causes an attenuation of the MMG signal due to restriction of muscle-fiber oscillations. Thus, the greater Pt potentiation of the young men may result in greater active muscle stiffness, which tended to minimize the MMG amplitude change compared with the old subjects, who have less Pt potentiation and a smaller increase in muscle stiffness.

**Electrically Evoked Torque and MMG Responses.** Although the Pt was the same for both groups at rest, the potentiation of Pt was significantly greater in the young than old men for 45 s after the MVC. This smaller degree of twitch potentiation observed in the elderly as compared to the young men confirms the findings of previous studies in the dorsiflexors. Our frequent assessments of Pt within the first minute narrows the window of time wherein the age-related difference in Pt disappears from a point between 5 s and 1 min to a time between 45 s and 1 min after the MVC. Of minor note, subjects experienced a greater degree of potentiation than their counterparts in the earlier two studies, an effect most likely due to our use of a longer-duration potentiation contraction.

**Voluntary EMG and MMG Responses.** The increase in surface EMG in relation to higher contractile intensities has been reported before in this and other muscles. Because of the many potential factors that can affect the surface EMG signal, comparison of surface EMG changes among individuals and between groups requires some form of normalization to be meaningful. In this study we normalized the surface EMG to the supramaximal M-wave for each subject. With this method, although there was a trend at low torque levels for the old adults to have greater relative EMG (Fig. 4A), the group effect was not statistically significant. Thus, there was no difference in the overall neural strategies employed by either group to grade torque. One other study compared changes in surface EMG with dorsiflexor torque and found marginally greater EMG only at the lowest torque values for old subjects compared with young adults, but the EMG was integrated and normalized to the peak MVC EMG. Had we normalized to the peak MVC EMG, it appears from Figure 4A that the EMG from the old subjects might have been statistically greater for the low torque levels. Regardless, our results suggest that any differences are marginal and would be limited to the lowest submaximal torques. Age-related MU remodeling, which suggests fewer but larger MUs with an overall loss of muscle mass as aging progresses, likely explains the smaller M-wave amplitude in the old subjects, and thus normalizing to this parameter may be a better method in studies on aging.

The MMG signal, like the EMG, is the product of MU activation strategy, i.e., MU recruitment and MU firing rates. Moreover, like the surface EMG, the MMG signal can be affected by several factors, including active muscle stiffness, neural activation strategies, adipose thickness, muscle fiber type.
composition, muscle mass, and muscle structure. It seems reasonable that the influence of some of these factors would vary among subjects and between groups. Accordingly, we suggest that to make more meaningful comparisons, the MMG should also be normalized. Therefore, as for the surface EMG, we normalized the MMG recorded during voluntary contractions of varying intensities to the MMG amplitude recorded during the supramaximal evoked twitch.

An increase in the MMG with increasing torque reflects greater neural activation. For the young adults, the normalized MMG signal showed only a slight increase at the lowest torque values, but then increased linearly by a factor of 4 between 40% and 100% MVC (Fig. 4B). A similar MMG–torque relationship was reported in the quadriceps and adductor pollicis, but not the biceps, in which the MMG declined beyond 65% or 80% of MVC. The relationship, however, was different for the old subjects, as there was almost no change between 20% and 60% MVC, a modest (~35%) increase of the normalized surface MMG signal between 60% and 80% MVC, and finally a slight (~10%) decrease at MVC. The decrease of the MMG signal at high torque levels is a previously observed phenomenon that has been attributed to a fusion of mechanical MU activity at the highest firing rates that restricts muscle-fiber oscillations. A greater degree of mechanical fusion may have been present in our old than young subjects because of the observed age-related slowing of contractile properties (Table 1). Alternatively, young subjects may continue to recruit MUs at a contraction level beyond that in the old, a suggestion that fits the premise that MU remodeling begins with the loss of the highest threshold units.

Although MMG was greater for young subjects at MVC, there was a trend for the MMG to be greater in the old subjects at the lowest torque levels (Fig. 4B). This finding is in partial contrast to the two earlier studies that examined the effect of age on the MMG–torque relationship, in which old men had absolute MMG that was smaller than that of young men in the elbow flexors at all levels of contraction intensity. Both of these earlier studies compared MMG across age groups in absolute units (m/s² and mV, respectively) without any method of normalization to account for potential differences in muscle composition and properties between adults of different age. Methodological issues may also have contributed to the difference in our results relative to the earlier studies. Esposito et al. employed MMG derived by sound rather than an accelerometer, and Akataki et al. used a ramped contraction that halted at 80% MVC rather than individual contractions at different intensities including MVC. In addition, Stokes and Dalton reported that the MMG–force relationship may be different in each muscle, depending on the fiber-type composition and distribution, and Yoshitake and Moritani suggested that the MMG–torque relationship may vary with overall muscle structure (biarticular vs. mono-articular muscle).

In summary, without a change in M-wave amplitude, the evoked MMG amplitude following a 10-s MVC was similar between young and old men, yet evoked twitch torque was significantly greater in young as compared to old men. Under voluntary conditions, both the normalized MMG–torque and EMG–torque relationships were similar between young and old men (except MMG at 100% MVC). However, whereas the MMG and EMG profiles were similarly shaped in the young men, they possessed different profiles in the old men. These results indicate that electrical and mechanical properties (during either electrically induced or voluntary contractions) are affected differently by the process of aging and that MMG can provide additional insight about neuromuscular function.

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ABSTRACT: Ultrasonography is a new imaging method for visualizing peripheral nerves. In vasculitic neuropathy, pain or axonopathy often prevent the lesion from being localized during electrophysiological examinations, but the ability of ultrasonography to evaluate it morphologically is unknown. Our aim was to determine whether ultrasonography could be used to detect abnormalities in tibial vasculitic neuropathy at the medial ankle. We evaluated 11 tibial nerves in 8 patients with tibial vasculitic neuropathy, and 35 tibial nerves in 35 control subjects. In the controls, the tibial nerve was successfully visualized as a hyperechoic nodule with multiple round hypoechoic areas transversely; in the patients, the tibial nerve appeared enlarged and hypoechoic. The affected nerve area was significantly larger (13.5 ± 3.7 mm²) than in controls (7.2 ± 1.5 mm²). Our results suggest that ultrasonography is a useful neuroimaging method for evaluation of tibial vasculitic neuropathy, especially when nerve conduction study findings are inconclusive.


ULTRASONOGRAPHY OF THE TIBIAL NERVE IN VASCULITIC NEUROPATHY

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Churg–Strauss syndrome, microscopic polyangiitis, Wegener’s granulomatosis, and polyarteritis nodosa are systemic vasculitides that commonly affect the epineurial vessels in the vasa nervorum and produce peripheral neuropathies.3 Nerve conduction studies (NCS) and electromyography are helpful for diagnosing peripheral neuropathies, but patients with vasculitic neuropathy often have painful sensory disturbances and may be intolerant of electrophysiological examinations. Further, severe axonopathy leading to absence of a recordable compound muscle action potential (CMAP) or sensory nerve action potential (SNAP) will lead to difficulties in localizing the exact site of the lesion.2,12 In contrast, ultrasonography (US) can be performed to assess peripher-
tibial nerve. In all of the patients, a diagnosis of tibial neuropathy was made after clinical examination, based on the following criteria: sensory disturbance in the toe, sole, or heel, or weakness of the flexor digitorum longus (FDL) and flexor hallucis longus (FHL) muscles, and a tibial nerve conduction study. Exclusion criteria were sensory disturbance radiating to the popliteal fossa, and a past history of entrapment neuropathy, polyneuropathy, or systemic disease other than vasculitis. Systemic vasculitis and clinically probable vasculitic neuropathy were diagnosed according to previously established criteria. For a diagnosis of tibial vasculitic neuropathy, both tibial neuropathy and vasculitis were required criteria. All subjects provided informed oral consent, and the study was undertaken with the permission of our ethics committee on clinical investigation.

Nerve Conduction Study. Tibial motor NCSs were performed using standard methods. The cut-off values for abnormality were as follows (mean – 2 SD): motor nerve conduction velocity of less than 43 m/s and abductor hallucis CMAP less than 7.4 mV.

Ultrasonographic Study. An experienced neurologist, who was blinded to the clinical and electrodiagnostic data, performed the sonographic examinations of the tibial nerve about 2 cm proximal to the top of the medial malleolus using a 7.5-MHz linear-array transducer (PLN-703AT probe, SSA-390A machine; Toshiba, Tokyo, Japan) using standard methods. Transversely, the nerve area was measured within the hyperechoic rim surrounding the nerve. Each measurement was taken three times, and the mean value was used in the analysis. From the measurements in our controls, the cut-off value for an abnormally large tibial nerve area was determined to be greater than 9.4 mm².

Statistical Analysis. Student’s t-test was used to compare the mean nerve area between patients and control subjects. P < 0.05 was considered statistically significant.

RESULTS

Clinical Features. Of the 13 affected tibial nerves identified in 8 patients, 2 were excluded from analysis because of sensory disturbance radiating to the popliteal fossa. With regard to the remaining 11 tibial nerves, sensorimotor signs were found in 2 cases, purely motor signs in 2, and purely sensory signs in 7; burning pain around the ankle occurred in all 9 with sensory signs.

Pathological Features. Biopsies were performed in all patients, except for patient 3. The organs biopsied were the skin (patients 2, 5, and 8), lungs (patients 1 and 7), and sural nerve (patients 4 and 6). Necrotizing vasculitis was found in lung (patients 1 and 7) and skin (patients 5 and 8) samples. In addition, granulomas were detected in lung tissues (patients 1 and 7), and extravascular eosinophils were observed in lung (patient 1) and skin (patient 2) tissues. The biopsy specimens also demonstrated extravascular cell infiltration (patient 4), and axonal degeneration and intrafascicular edema (patient 6).

Nerve Conduction Studies. Motor NCS was attempted for 11 tibial nerves, but intolerable pain in 3 instances required stoppage of the examination. Abductor hallucis CMAPs were unrecordable from 2 nerves and were less than 7.4 mV in 6 nerves, which is compatible with an axonopathy.

Ultrasonographic Features. In the controls, the tibial nerve appeared as a hyperechoic nodule, with multiple round hypoechoic areas transversely and a hyperechoic structure with parallel linear echoes longitudinally, whereas in the affected patients the tibial nerve appeared as enlarged and hypoechoic. Proximal to the lesions, the tibial nerves showed normal echo texture and size (Fig. 1). The mean cross-sectional area of the tibial nerve at the ankle in the eight patients was significantly larger (13.5 ± 3.7 mm²; range, 9.3–19.8 mm²) than in controls (7.9 ± 1.5 mm²; range, 5.0–10.7 mm²) (P < 0.05).

The results of US were compared to the NCS. Nerve enlargements were detected by US in 10 nerves, for which NCS suggested an axonopathy in 7 and were inconclusive in 3; these 3 were intolerant of the NCS. Further, a normal area was detected by US for 1 nerve from which no recordings could be obtained during NCS. No adverse events were reported during the US examinations.

DISCUSSION

We found that the mean tibial nerve area in patients with tibial vasculitic neuropathy was significantly larger than in control subjects. Therefore, we believe that this nerve enlargement probably reflects pathophysiologic changes occurring in the peripheral nerves. We hypothesize that a vasculitic–granulomatous lesion in the epineurium, as well as intrafascicular edema as a consequence of nerve ischemia and axonal degeneration, are responsible for such localized nerve enlargement based on pathological findings, although the pathogenesis of nerve enlarge-
ment in tibial vasculitic neuropathy is uncertain because the affected tibial nerve has rarely been biopsied.8 Granuloma or intrafascicular edema, as seen in our patients, has occasionally been reported in the enlarged nerves of neurosarcoidosis,5 leprosy,11 chronic inflammatory demyelinating polyradiculoneuropathy,9 and hypertrophic mononeuritis.8

Our results indicate the nerve enlargement that occurred at the ankle. In general, vasculitic mononeuritis multiplex more often involves the legs than the arms, and the distal nerves more than proximal nerves.3 Additionally, all patients who were selected from inclusion criteria in the present study had burning pain around the ankle. Therefore, we speculate that the nerve enlargement at the medial ankle is caused by active vasculitic lesions. The present study is limited by the small number of subjects, because tibial vasculitic neuropathy is relatively uncommon.

In conclusion, US is a useful neuroimaging method for the evaluation of localized nerve enlargement at the ankle in tibial vasculitic neuropathy associated with systemic vasculitis, especially when NCS results are inconclusive. Further studies in patients with tibial vasculitic neuropathy are needed to confirm our findings and may add to understanding of the pathophysiology of tibial vasculitic neuropathy.

This study was presented in part at the 57th annual meeting of the American Academy of Neurology, April 2005, Miami Beach, Florida.

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FIGURE 1. Ultrasonographic images from a normal control (A, C) and a patient with tibial vasculitic neuropathy (B, D). In the normal control, the tibial nerve in a transverse section view (A) (circled by dots) can be seen accompanied by the posterior tibial artery (A) (asterisk) and appears as a hyperechoic nodule with multiple round hypoechoic areas inside; in a longitudinal section view (C) (arrows) it appears as a hyperechoic structure with parallel linear echoes, a so-called “tram-track” appearance. In tibial vasculitic neuropathy (patient 1), the tibial nerve in a transverse section view (B) (circled by dots) is seen as enlarged, and in a longitudinal section view (D) (arrowheads) appears as enlarged, blurred, and hypoechoic. Proximal to the lesion (left side of panel), the tibial nerve (D) (arrows) appears to have normal echo texture and size. An upward flow approaching the echo probe is indicated by the red and a downward flow is indicated by the blue. Scale bar, 5 mm.
ABSTRACT: We examined the role of needle electromyography (EMG) of the rectus abdominis (RA) in assessing thoracic involvement in amyotrophic lateral sclerosis (ALS). Needle EMG of the RA was performed in 67 patients with sporadic ALS and 110 healthy controls. The presence of abnormal spontaneous activity, configuration of motor unit action potentials (MUAPs), and recruitment pattern of motor unit potentials were examined. In ALS patients, MUAPs in the RA were of prolonged duration, large amplitude, and showed increased prevalence of polyphasic waveforms compared to controls. Significant differences in MUAP parameters, presence of abnormal spontaneous potentials, and interference patterns were noted between ALS patients and controls. Additionally, we found that active denervation was more frequent in the RA of ALS patients with dyspnea than those without dyspnea. Thus, conventional needle EMG of the RA is a valuable electrophysiological method to assess clinical and subclinical involvement of thoracic lower motor neurons in patients with suspected ALS.


NEEDLE ELECTROMYOGRAPHY OF THE RECTUS ABDOMINIS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder affecting motor neurons of the cerebral cortex, lower brainstem, and spinal cord. Many ancillary techniques have been used to detect clinical or subclinical lesions of these regions. Electromyography (EMG) is helpful in identifying loss of lower motor neurons in ALS, and documenting its progression, thus minimizing the occurrence of misdiagnosis. EMG of the lower thoracic paraspinal muscles may detect lesions of the thoracic region, but isolation and evaluation of motor unit action potentials (MUAPs) in the thoracic paraspinal muscle may be difficult due to insufficient or excessive paraspinal muscle activity. We therefore explored the use of EMG of the rectus abdominis (RA) as an alternative for assessing thoracic involvement in the diagnosis of ALS.

Abbreviations: ALS, amyotrophic lateral sclerosis; EMG, electromyography; MUAP, motor unit action potential; RA, rectus abdominis

Key words: ALS; amyotrophic lateral sclerosis; electromyography; rectus abdominis; thoracic region

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METHODS

Subjects. We evaluated 110 healthy individuals (23–77 years; mean age 45.4 ± 8.3 years; 60 men and 50 women), and 67 patients with clinically definite, sporadic ALS, according to the El Escorial criteria (25–67 years; mean age 43.5 ± 7.3 years; 46 men and 21 women). Patients’ disease duration ranged from 6 months to 36 months (mean, 20 ± 3 months). Symptoms of respiratory dysfunction, such as dyspnea, were noted in 19 ALS patients, and 3 patients were on noninvasive positive pressure ventilation. Our institutional ethical committee approved the study. The study design was fully explained and written information was provided to each subject, all of whom signed an informed consent form.

Electrophysiological Study. EMG was performed with a concentric needle electrode at standard settings (Keypoint; Medtronic, Skovlunde, Denmark). Care was taken to ensure complete relaxation during evaluation of spontaneous activity. MUAP configuration was assessed during voluntary activity. At least 20 MUAPs were measured in each muscle. Parameters were calculated using a quantitative EMG mode. MUAP recruitments were assessed subjectively dur-
ing maximal voluntary effort. EMG of the RA was performed above the umbilicus. Contracting RA can be seen as bulgings between tendinous intersections in subjects who are not overweight. The needle was inserted approximately 1 cm lateral to the midline, where the second or third belly lies. EMG of the lower thoracic paraspinal muscle was performed at T10; the needle was inserted to the bony lamina, approximately 2–3 cm lateral to the midline, and then withdrawn a few millimeters so as to be in the deep paraspinal muscles. Given that the voluntary effect of this muscle is hard to control, only spontaneous activity and recruitment patterns were observed in the lower thoracic paraspinal muscle.

**Statistical Analysis.** Independent *t*-tests, paired *t*-tests, one-way repeated-measures analysis of variance (ANOVA), and chi-square tests were used. A value of *P* ≤ 0.05 was considered significant.

**RESULTS**

In the RA of 110 healthy controls, the MUAP parameters obtained are shown in Table 1. There were no statistical differences in parameters based on age, left- or right-sided measurements, or gender among the healthy controls, nor were abnormal spontaneous potentials found in these subjects. In comparison, 63 patients with ALS had a neurogenic EMG, whereas 4 patients had a normal EMG. Among those with neurogenic findings, 17 showed asymmetric findings, which were normal on one side and abnormal on the other side. The MUAP characteristics in the 67 ALS patients are shown in Table 1 and were significantly different from control values. Additionally, in ALS patients, abnormal spontaneous potentials were found in the RA in 50 cases (75%), and a reduced interference pattern was found in 63 cases (94%).

EMG of the lower paraspinal muscles showed abnormal spontaneous potentials and incomplete interference patterns in 57 ALS patients (85%). No significant differences in EMG neurogenic changes were noted between the RA and the lower paraspinal muscles in patients with ALS (63 vs. 57 cases; 94% vs. 85%, *P* > 0.05). Comparison of subgroups of ALS patients with (*n* = 19) and without respiratory dys-

<table>
<thead>
<tr>
<th>MUAPs</th>
<th>Controls (n = 110)</th>
<th>ALS patients (n = 67)</th>
<th><em>P</em>-value</th>
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</thead>
<tbody>
<tr>
<td>Duration (ms)</td>
<td>9.95 ± 1.13</td>
<td>13.02 ± 1.30</td>
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<tr>
<td>Amplitude (µV)</td>
<td>373.78 ± 56.46</td>
<td>537.19 ± 159.04</td>
<td>0.003</td>
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<tr>
<td>Polyphasic potentials (%)</td>
<td>11.75 ± 3.26</td>
<td>31.19 ± 8.84</td>
<td>0.001</td>
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**DISCUSSION**

We found that neurogenic changes in the EMG of the RA muscle may indicate lesions of the lower motor neurons within the thoracic region of the spinal cord, and suggest that evaluation of the RA may be helpful in the diagnosis of ALS. Additionally, an asymmetric EMG on the two sides may suggest an early stage of the disease.

ALS is most reliably identified when clinical and EMG changes are found diffusely, i.e., in several distinct anatomical regions. However, there is presently no consensus about the best means of localizing lesions in the thoracic region, and there have been reports of the inadequacy of EMG of the paraspinal muscle in this regard.

The RA lies within the anterior abdominal wall, beside the anterior median line (linea alba), and the anterior branches of the T5–12 spinal nerves innervate the muscle. There are four to five bellies (each 1-cm thick) in this muscle, divided by tendinous intersections. EMG of the RA was obtained by insertion of bipolar electrodes into the belly just above the umbilicus, which was chosen because the fat in the lower umbilical region is thicker.

We found no differences between the EMG of the RA and the lower thoracic paraspinal muscle. It is more convenient, however, to perform EMG of the RA than the paraspinal muscle. First, the RA is a safe muscle for EMG examination. Second, RA EMG requires the patient to be in a supine position whereas the paraspinal muscle utilizes the prone position, which is inconvenient for ALS patients, especially for those with dyspnea. Third, it is easy for RA to be used to measure voluntary effort.

It is relevant to note that the RA is an important respiratory muscle, and respiratory muscle involve-
ment can be detected in most patients before respiratory symptoms begin. Respiratory failure is a common cause of death in patients with ALS. Clinical manifestations are useful in recognizing respiratory dysfunction (patients with a forced vital capacity less than 50% of the predicted value are at risk of respiratory compromise), but often occur late. The electrophysiological examination may play an important role in diagnosing the cause of dyspnea. Some studies have demonstrated that the diaphragm takes part in the inspiratory process whereas the RA is mainly an expiratory muscle. Ortega et al. demonstrated the relationship between the respiratory status of chronic obstructive pulmonary disease and the surface EMG of the RA. In this study, we found that the percentage of ALS patients having an abnormal EMG of the RA was greater in those with than without dyspnea, suggesting involvement of respiratory function, but this requires further study in a large group of patients.

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ABSTRACT: In desmin myopathy but not hereditary inclusion-body myopathy (hIBM), there is accumulation of myofibrillar proteins including desmin, myotilin, dystrophin, gelsolin, actin, and CDC kinase. To assess the cause of protein excess, we studied the genes coding the accumulated proteins in desmin myopathy, hIBM, and controls. No differences were found among them. In desmin myopathy, protein accumulation is not due to upregulation of genes triggered by mutant desmin, but rather to posttranslational disassembly of intermediate filaments.


ABSENCE OF UPREGULATED GENES ASSOCIATED WITH PROTEIN ACCUMULATIONS IN DESMIN MYOPATHY

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Myofibrillar myopathy is a heterogeneous group of myopathies histologically characterized by deposits of amorphous, granular, or hyaline material; small vacuoles; and disorganization of the Z disks owing to disruption of intermediate filaments.9 Among the group of myofibrillar myopathies, desmin myopathy is a distinct subset caused by pathogenic mutations in the desmin gene.3,6 Similar disease, clinically and histologically, is also caused by mutations in αB crystallin, myotilin, and ZASP.5,12–14 In all these genetically defined myopathies, the respective mutations result in accumulation within the myofibers of various myofibrillar or intermediate filament proteins such as myotilin, desmin, dystrophin, neural cell adhesion molecule (NCAM), cell division cycle (CDC) kinase 2, gelsolin, beta-amyloid precursor protein (βAPP), prion, plectin, and actin.6,12–14 These accumulations have prompted the designation “protein-surplus myopathy.”75 It is unclear, however, whether the excessive protein deposition within the myofibers is due to upregulation of the respective genes or due to disassembly of the intermediate filaments and disruption of myofibrillar integrity in degenerating fibers. Such information is useful in understanding the mechanisms that dictate protein accumulation and their significance in the cause of muscle weakness.

We report the gene expression levels of the proteins accumulated in muscles of patients with desmin myopathy compared to normals and disease controls who had a similar myopathy but without protein accumulation in the myofibers.

MATERIALS AND METHODS

Patients. Muscle biopsies were obtained from four patients with desmin myopathy, three with hereditary inclusion-body myopathy (hIBM), and two normal controls. The diseases were clinically and histologically characterized, as previously described.3,7 The patients with hIBM due to GNE mutations were selected as the closest disease control because they have a hereditary vacuolar myopathy with a genetic defect,4 present with distal and proximal muscle involvement as seen in desmin myopathy, and possess some protein accumulations within the myofibers.1

Microarray and Data Filtration. Total RNA from muscle biopsies was reverse transcribed (Trizol; Invitrogen, Carlsbad, California) and biotinylated cRNA probes were generated by in vitro transcription (Ambion, Austin, Texas). Fragmented cRNA (15 µg) was hybridized to a human genome U133A array containing 22,283 oligonucleotide probe sets representing transcripts derived from approximately 16,000 human genes (Affymetrix, Santa Clara, California). The hybridized gene chips were scanned to quantify...
the gene expression and the data analysis was performed using a microarray suite and data mining tool (Affymetrix). The data, normalized by GeneSpring (Silicon Genetics, Foster City, California) software, was initially selected for genes that had “present” or “marginal” calls in 50% or more samples. All samples were coded and analyzed blindly. The mean fold difference of expression of the selected genes between controls and disease groups (desmin myopathy and hIBM) was calculated.

When microarray experiments using the two normal controls were duplicated in separate muscle sections by isolating RNA and performing hybridization separately from each section, we found excellent correlation. Further, after normalization, the correlation coefficient for the two independently performed microarray experiments from the same subjects were 0.97 and 0.96 for controls 1 and 2, respectively.

**Real-Time Polymerase Chain Reaction.** The microarray data were selectively corroborated with real-time polymerase chain reaction (PCR) experiments. TaqMan primer/probe sets were purchased from Applied Biosystems (Santa Clara, California) and the protocol used was the one provided by the manufacturer. Briefly, RNA was isolated, reverse transcribed, and cDNA equivalent to 1 ng RNA was used per tube for amplification. Real-time PCR was performed using an Opticon II thermocycler (MJ Research, Waltham, Massachusetts). Expression levels of two representative genes, desmin and αB crystallin, were tested by real-time PCR on the muscle biopsies of desmin myopathy and controls. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

**Immunohistochemistry.** In order to compare the microarray data with the protein expression, we performed immunohistochemistry to immunolocalize desmin as a representative gene product in the muscle biopsies of patients with desmin myopathy. We employed trichrome staining and standard immunofluorescence technique using antidesmin antibodies on consecutive fresh-frozen muscle biopsy sections as previously described.

**RESULTS**

As shown in Table 1, the genes corresponding to most of the proteins reported to be accumulated in the muscles of patients with desmin myopathy, such as myotilin, desmin, plectin, αB crystallin, dystrophin, NCAM, CDC2 kinase, gelsolin, titin, βAPP, prion, α1-antichymotrypsin, and actin, remained unaltered, as compared to the genes seen in the disease control muscles. We found a significant (more than twofold) increase in the expression of genes corresponding to only two of these proteins, gelsolin and α-actin. However, the muscles of hIBM patients demonstrated much more upregulation of the mRNA levels of these genes (Table 1). In reference to other genes, 16 genes in desmin myopathy were upregulated more than threefold and 202 genes more than twofold, compared to controls, but these changes were also noted in hIBM. Similarly, genes that were down-regulated in desmin myopathy were also down-regulated in hIBM. In contrast, a large number of genes found to be distinctly altered in hIBM, com-
pared to controls, remained unchanged in desmin myopathy muscle (data not shown).

Immunohistochemistry confirmed the protein accumulation of desmin in desmin myopathy muscle, even though the mRNA expression levels of desmin were not altered (Table 1). By real-time PCR, amplification of desmin and αβ crystallin in the same muscle did not show a difference in the level of mRNA expression between desmin myopathy and controls.

DISCUSSION

Using oligonucleotide microarrays, we found that genes coding most of the proteins that aberrantly accumulate in the muscle fibers of patients with desmin myopathy are not upregulated or altered compared to hIBM, where no accumulations of myofibrillar proteins are observed. To the contrary, genes coding several of these proteins, such as α-actin and gelsolin, were more upregulated in hIBM than desmin myopathy. This finding indicates that the accumulation of proteins seen in desmin myopathy and other myofibrillar myopathies is not due to alteration of expression of the respective genes but rather due to a posttranslational physical disassembly of the intermediate filaments and disruption of myofibrillar integrity in the degenerating fibers. This physical disruption, as reported earlier, may be due to alterations in protein solubility that result in deposition of insoluble aggregates.8

Desmin plays an essential role in maintaining muscle cytoarchitecture by forming a three-dimensional scaffold around the myofibrillar Z disk and by connecting the entire contractile apparatus to the subsarcolemmal cytoskeleton, the nuclei, and other organelles. It appears that a mutation in any one of these proteins within the myofibrillar network, such as desmin, αβ crystallin, myotilin, or the other Z-disk proteins, can induce the collapse of the filamentous network and trigger a pathogenic cascade of protein aggregation. Such protein aggregates probably linked to reduced solubility and degradation, may be deleterious to the cell because they can impair the function of the ubiquitin–proteasome system, stressing the myofiber, and result in further accumulation of other proteins, as observed with mutated αβ crystalline.10,15 The term protein-surplus myopathy, as proposed,5 does not therefore accurately reflect the pathogenic cause of the myopathy because there is no active process at the gene level that leads to formation of protein aggregates.

The observation that intermediate filament aggregates within the myofiber are the result of their mechanical disassembly caused by mutation in one of the proteins, but not by activation of the respective genes, may be useful in understanding the role of intermediate filament protein accumulation in other neurodegenerative disorders caused by mutations in cytoskeletal proteins. Mutations in neuronal intermediate filaments, for example, have been associated with alteration of expression, function, or biochemistry of these proteins, which aggregate and form potentially toxic inclusions within the neuronal cells in patients with Alzheimer’s disease, amyotrophic lateral sclerosis, Charcot–Marie–Tooth disease, and some other neuropathies.2

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CASE OF THE MONTH

ABSTRACT: We report three patients with anti–signal recognition particle antibodies who had different presenting clinical pictures, mimicking acute polymyositis, limb-girdle muscular dystrophy, and acute rhabdomyolysis. Muscle biopsies typically showed necrotizing myopathy with little or no inflammation and deposits of membrane attack complex (C5b-9) in endomysial capillaries. The clinical course was severe in two patients and mild in one. The combination of corticosteroid with either an immunosuppressive agent or intravenous immunoglobulins was required to improve the condition of these patients.


MYOPATHY ASSOCIATED WITH ANTI–SIGNAL RECOGNITION PEPTIDE ANTIBODIES: CLINICAL HETEROGENEITY CONTRASTS WITH STEREOTYPED HISTOPATHOLOGY

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Anti–signal recognition particle (SRP) antibodies are myositis-specific antibodies usually found in 4%–6% of patients with idiopathic inflammatory myopathies (IIM).1,8,11–13.17.20 Patients with anti-SRP antibodies most often present with severe muscle involvement characterized by both rapidly developing proximal weakness culminating in severe disability, and poor response to steroid.8,11–13.20 Anti-SRP patients were usually classified as having polymyositis (PM).8,11,12.20 However, a recent study pointed out that anti-SRP myopathy has distinct stereotyped histopathological features including: (1) an active necrotizing myopathy; (2) no or little inflammation; and (3) an endomysial microangiopathy assessed by membrane attack complex (C5b-9) deposition and capillary loss.13 We report three patients with different clinical presentations, but highly similar features at muscle biopsy and positive detection of anti-SRP antibodies. Since anti-SRP myopathy appears clinically polymorphic, we believe that recognition of its peculiar pathological features may allow early diagnosis and treatment, and therefore could improve the overall prognosis.

CASE REPORTS

Patient 1. A 27-year-old French-Caribbean woman was admitted to Henri Mondor Hospital in December 2002 with a 1-month history of rapidly progressive severe weakness in all four limbs. She had a history of asthma, erythema nodosum in 1993, and three spontaneous abortions in 2001 and 2002. She presented with mild generalized myalgia and moderate dysphagia. Examination showed severe symmetrical weakness of grade 3 in the deltoid and 2 in

Abbreviations: ANA, antinuclear antibody; CK, creatine kinase; CRP, C-reactive protein; DM, dermatomyositis; EMG, electromyography; ESR, erythrocyte sedimentation rate; FITC, fluorescein isothiocyanate; IIM, idiopathic inflammatory myopathy; IMNM, immune-mediated necrotizing myopathy; IVIg, intravenous immunoglobulin; LGMD, limb-girdle muscular dystrophy; MAC, membrane attack complex; MHC, major histocompatibility complex; MRC, Medical Research Council; NCAM, neural cell adhesion molecule; PAS, periodic acid–Schiff; PM, polymyositis; SG, sarcoglycan; SRP, signal recognition peptide

Key words: anti–signal recognition peptide antibodies; anti–signal recognition peptide myopathy; anti–signal recognition peptide immune-mediated necrotizing myopathy

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the iliopsoas muscles, according to the Medical Research Council (MRC) scale. There were no other motor deficits. Skin, sensory, stretch reflex, and cardiopulmonary examinations were normal. Serum creatine kinase (CK) was 9665 IU/L (normal, <185 IU/L). Electromyography (EMG) showed short-duration, small-amplitude, polyphasic motor unit potentials in the deltoid and supinator muscles without fibrillation potentials. Routine laboratory tests (see below), including complete blood cell count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and cardiopulmonary investigations, were normal. Deltoid muscle biopsy showed an active necrotizing myopathy without conspicuous inflammation. Serum antinuclear antibody (ANA) was negative but a particular cytoplasmic staining pattern on HEp-2 cells (1:1280) led to the identification of anti-SRP antibodies. Intravenous methylprednisolone (1 g daily for 5 days) was followed by oral prednisone (1.5 mg/kg daily). Two months later, methotrexate (20 mg/week) was added because of insufficient improvement. She improved markedly with a parallel decrease of serum CK to below 500 IU/L. Prednisone was progressively tapered at 7 mg/day and methotrexate was stopped in October 2005. In January 2006, muscle strength and serum CK were nearly normal.

Patient 2. A 31-year-old Malian man was admitted to Saint Camille Hospital in 2004 for a disabling chronic myopathy. In 1997, he was first evaluated in Spain for progressive limb weakness, and initially diagnosed as having PM. Weakness worsened over 3 years despite several therapeutic approaches, including steroids, intravenous immunoglobulins (IVIg), and azathioprine. Finally, the patient was classified as having limb-girdle muscular dystrophy (LGMD). On admission, he was unable to walk and complained of mild myalgia. Examination showed severe symmetrical weakness of grade 2 in the deltoid muscles, 4 in the wrist extensors, 2 in the iliopsoas and the quadriceps muscles, and 3 in the neck flexors. Sensory, stretch reflex, skin, and cardiopulmonary examinations were normal. Serum CK was 3660 IU/L. EMG showed short-duration, small-amplitude, polyphasic motor unit potentials in the deltoid, biceps, supinator, and quadriceps muscles, without fibrillation potentials. Routine laboratory tests (see below) and cardiopulmonary investigations were normal. Deltoid muscle biopsy showed an active necrotizing myopathy with endomysial fibrosis. Serum ANA was negative but the particular cytoplasmic staining pattern on HEp-2 cells (1:1280) led to the identification of anti-SRP antibodies. He was treated from October 2004 with prednisone (1 mg/kg daily) and six monthly IVIg infusions (2 g/kg), and in January 2005 methotrexate (30 mg/week) was added. He improved markedly and became able to walk without aid. Serum CK declined to 321 IU/L. In October 2005, he presented with a flare and raised serum CK at 1103 IU/L while on prednisone 25 mg/day and methotrexate 30 mg/week. Reinstitution of monthly IVIg therapy from November 2005 with a tapering dose of prednisone (20 mg/day) induced clinical improvement with a parallel decrease of serum CK to approximately 500 IU/L.

Patient 3. A 46-year-old Senegalese man was admitted to Saint Camille Hospital in 1998 with rhabdomyolysis (serum CK, 21,600 IU/L). Examination showed only mild proximal weakness (MRC grade 4) in the deltoid muscles. Sensory, stretch reflexes, skin, and cardiopulmonary examinations were normal. EMG showed short-duration, small-amplitude, and polyphasic motor unit potentials in the deltoid muscles, without fibrillation potentials. Routine laboratory tests (see below) and cardiopulmonary investigations were normal. Deltoid muscle biopsy showed moderate necrotizing myopathy with minute lymphocytic infiltrates. Serum ANA was negative but a particular cytoplasmic staining pattern on HEp-2 cells (>1:1280) led to the identification of anti-SRP antibodies. Prednisone was started in July 1998 (1.5 mg/kg per day) with complete clinical recovery, but serum CK remained elevated, ranging from 2000 to 6000 IU/L. In November 1998, when prednisone was tapered to 40 mg/day, his condition worsened, with strength in the deltoid and pelvic-girdle muscles at grade 4; serum CK increased to 12,580 IU/L. He was treated with prednisone (1 mg/kg per day) for 3 months, and methotrexate (15 mg/week) was added in February 1999. He improved markedly, with strength being nearly normal and serum CK declining to 500 IU/L. In April 2000, prednisone was tapered to 20 mg/day, but he developed myalgia without weakness and a serum CK of 923 IU/L. From April to August 2000, he received five IVIg infusions (2 g/kg). He improved again and serum CK decreased to below 1000 IU/L. Prednisone was progressively tapered to 5 mg/day and methotrexate was stopped in July 2002. He remained stable and serum CK varied between 1000 and 2000 IU/L over the next 4 years. In September 2004, his condition worsened again and serum CK increased to 7000 IU/L. Prednisone (0.5 mg/kg per day), methotrexate (30 mg/week), and monthly IVIg led to improvement, and his condition has since remained stable.
Muscle Biopsy. Muscle samples were conventionally processed for light microscopy using standard procedures. Frozen and paraffin-embedded sections were stained using hematoxylin–eosin (H&E), Mason and modified Gomori trichrome, Sudan black, periodic acid–Schiff (PAS), and histoenzymatic reactions including nicotinamide adenine dinucleotide (NADH)–tetrazolium reductase, succinate dehydrogenase, cytochrome c oxidase, myophosphorylase, and phosphofructokinase. Expressions of major histocompatibility complex (MHC)–1 (HLA-A, -B, -C) and –2 (HLA-DR), membrane attack complex (MAC)/C5b-9, CD3, CD4, CD8, CD20, CD56/neural cell adhesion molecule (NCAM), CD68, CD138, fast myosin, dystrophin (Dys-1, Dys-2, and Dys-3) α-sarco-glycan (SG), β-SG, γ-SG, δ-SG, dysferlin, caveolin-3, and merosin (Novocastra, Newcastle upon Tyne, UK) were evaluated by immunoperoxidase assay performed on frozen sections using an automated immunostainer (Ventana, Tucson, Arizona).

In all patients, muscle biopsy showed an active necrotizing myopathy, characterized by the presence of numerous necrotic and regenerating fibers at various stages of injury, including hyalinized fibers, myophagocytosis, basophilic fibers, and fibers with central nuclei (Fig. 1). In patients 1 and 2, the necrotic process was associated with angulated (patient 1) or rounded (patient 2) fiber atrophy, but without elective distribution: in particular, neither perifascicular atrophy nor focal myofilament loss was
observed. NCAM isoforms were widely expressed, indicating the larger number of regenerating fibers (Fig. 1G). MAC/C5b-9 dotted deposits were observed on the sarcolemma of several non-necrotic fibers (Fig. 2F–H). Expression of MHC-1 was lacking (patient 1) or occasional (patients 2 and 3) (Fig. 2A and B). In patient 3, endomysial connective tissue proliferation was observed in keeping with the protracted course of the disease (Fig. 1E). Inflammatory infiltrates were moderate, contrasting with the extent of necrosis, and consisted mainly of lymphocytic septal perivascular cuffs. In patients 2 and 3, rare infiltrates were observed in the endomysium, but not related to fiber injury. Moreover, focal attack or

**FIGURE 2.** Muscle biopsy, frozen sections, with immunoperoxidase stain. Patient 1 (A, C, D, F, G) and patient 3 (B, E, H). Staining of major histocompatibility complex class 1 (MHC-1) antigens shows distinct expression patterns. (A, B) Lack of staining of non-necrotic fibers in patient 1 (A) contrasts with presence of significant positivity in patient 3 (B). C5b-9 deposits (arrows) in endomysial capillaries (C–E). Sarcolemmal C5b-9 deposits (arrowheads) in non-necrotic fibers (F–H).

**FIGURE 3.** Indirect immunofluorescence pattern of anti-SRP antibodies on HEp-2 cells (×400).
invasion of non-necrotic fibers was absent. In all patients, biopsy disclosed endomysial microangiopathy, characterized by enlargement of capillary lumens, focal capillary depletion, and endothelial MAC/C5b-9 deposition (Fig. 2C–E). Sarcolemmal expressions of dystrophin, sarcoglycan subcomplex components, merosin, dysferlin, and caveolin-3 as well as enzyme activities were normal in non-necrotic fibers.

**Laboratory Testing.** Standard biochemistry, complete blood cell count, ESR, and CRP were normal in all patients. Serological tests for human immunodeficiency virus, human T-cell leukemia virus, and hepatitis C virus were negative. Serological tests for hepatitis B virus were negative or suggestive of previous immunization. Cardiopulmonary investigations included electrocardiogram, echocardiography, chest radiography, and high-resolution thoracic computed tomography scan, and these were normal in all patients.

Indirect immunofluorescence was performed using HEp-2 cells (Kallestad; Bio-Rad Laboratories, Redmond, Washington) and fluorescein-labeled anti-human immunoglobulin (Kallestad; Universal FITC conjugate). The specificity for anti-SRP was made with an immunodot assay using as antigen a preparation of native SRP extracted from dog pancreas obtained from Prof. Bernhard Dobberstein (ZMBH, Heidelberg, Germany). This dot assay was found to be highly specific for anti-SRP antibodies as no reaction was seen with antibodies of other specificities, such as anti-ribosome, anti-Jo1, PL7, and PL12, which also show a cytoplasmic staining on HEp-2 cells.

The three patients’ sera showed a diffuse, dense, fine granular cytoplasmic staining pattern of HEp-2 cells without nuclear or nucleolar fluorescence (≥1: 1280) (Fig. 3). Anti-SRP antibodies were identified in all three cases. Tests for antibodies to the aminocyl-tRNA synthetases (Jo-1, PL-7, and PL-12) and the ribosomal P proteins were negative in all patients.

**DISCUSSION**

IIM are classified according to their clinical and pathological features and are basically divided into three major conditions: PM; dermatomyositis (DM); and sporadic inclusion-body myositis. In the recent classification from the European Neuromuscular Consortium, two other categories were proposed: non-specific myositis and immune-mediated necrotizing myopathy (IMNM). Some of these IIM may be associated with so-called myositis-specific antibodies, the most common being anti-Jo-1 antibodies.

To date, the clinical features of anti–SRP-associated myopathy have been described in 65 patients (Table 1) and pathological features in 41 patients. Interestingly, in the current study, only patient 1 exhibited a rapidly developing and severe proximal weakness with markedly raised serum CK. In two patients, the clinical presentation was misleading, with chronic involvement mimicking LGMD in patient 2, and rhabdomyolysis contrasting with painless mild weakness in patient 3. None of our patients had clinical or laboratory signs of systemic disease, or cardiopulmonary involvement as usually reported. In contrast to the anti-synthetase syndrome, fever, arthritis, Raynaud’s phenomenon, and overlap syndromes are uncommon.11,12,14 In recent series, the mortality rate among anti-SRP patients did not differ from that observed in patients with other IIM.11,13 Association with malignancy is rare.8 The predominance of black patients suggests possible genetic determinants.12,17 A recent study did not confirm the seasonal occurrence of anti-SRP myopathy reported in early series.17

Major hyperCKemia (>3000 IU/L) and pathological features are common denominators for anti-SRP patients.1,8,11,15 In our patients, as in those reported by Miller et al., the prominent abnormal pathological feature consisted of many randomly distributed necrotic and regenerating muscle fibers. Inflammation was restricted to sparse cells or small perivascular cuffs, without perimysial infiltrates. In most cases, MHC-1 expression was absent, and, if present, it was usually weak and focal. Deposition of the terminal C5b-9 components of complement in endomysial capillaries, reduction in capillary density, and enlargement of capillary lumens were somewhat similar to vascular changes in DM.5,10 However, in our patients, C5b-9 deposits were irregular, punctuated, and less conspicuous than in DM. It must be noted that patients with anti-SRP myopathy may rarely present with a DM-like rash.4,8,18 Another peculiar observation was the presence of C5b-9 deposits along the sarcolemma of non-necrotic fibers, as reported in DM and lupus myopathy, but also in dystrophies.15,19 Endomysial fibrosis was observed when biopsy was performed late in the disease course. However, the increase in connective tissue can be marked despite a short duration (few months) of disease.13

Anti-SRP patients were first described as PM patients.4,9,11,12,20 However, lack of both conspicuous inflammation and expression of MHC-1 is an impor-
tant feature distinguishing anti-SRP myopathy from PM and other IIM. Such findings are rather misleading and usually lead to considerations of other diagnoses such as endocrine or toxic myopathy, or a muscular dystrophy such as dysferlinopathy. According to the classification of the European Neuromuscular Consortium, anti-SRP myopathy fulfills the diagnostic criteria of IMNM.\textsuperscript{10} To date, it is not established whether anti-SRP myopathy represents a specific entity or simply a subset of IMNM.\textsuperscript{3,6}

Detection of circulating anti-SRP antibodies is based upon indirect immunofluorescence showing a suggestive cytoplasmic pattern on HEp-2 cells. The SRP specificity must be confirmed by immunoprecipitation or by an immunodot assay with pure native SRP protein.

SRP is an ubiquitous cytosolic molecular complex made of six polypeptides and a small RNA molecule.\textsuperscript{16} SRPs bind growing polypeptides and drive their translocation into the endoplasmic reticulum lumen to allow achievement of protein synthesis. The significance of anti-SRP antibodies is unknown. Although occasionally detected in patients with systemic sclerosis without myopathy, anti-SRP antibodies are regarded as markers for necrotizing myopathy.\textsuperscript{8,11,13} The histological pattern is sufficiently stereotyped to indicate that an association between necrotizing myopathy and anti-SRP antibodies is not fortuitous. The immune-mediated pathogenic mechanisms underlying anti-SRP myopathy are as yet unknown.

### Table 1. Clinical characteristics of patients with anti-SRP myopathy\textsuperscript{,8,11–13,20}

<table>
<thead>
<tr>
<th></th>
<th>Targoff et al.\textsuperscript{20}</th>
<th>Love et al.\textsuperscript{12}</th>
<th>Miller et al.\textsuperscript{13}</th>
<th>Kao et al.\textsuperscript{11}</th>
<th>Hengstman et al.\textsuperscript{8}</th>
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<tbody>
<tr>
<td>Number of cases</td>
<td>12</td>
<td>7</td>
<td>7</td>
<td>16</td>
<td>23</td>
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<tr>
<td>Mean age at onset (range)</td>
<td>36 (19–59)</td>
<td>37</td>
<td>48 (32–70)</td>
<td>51 (25–78)</td>
<td>48</td>
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<tr>
<td>Gender</td>
<td>7 F/5 M</td>
<td>6 F/1 M</td>
<td>5 F/2 M</td>
<td>8 F/8 M</td>
<td>13 F/5 M</td>
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<td>ND</td>
<td>5</td>
<td>ND</td>
<td>4</td>
<td>ND</td>
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<td>Muscle weakness</td>
<td></td>
<td></td>
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<tr>
<td>Proximal &gt; distal</td>
<td>12</td>
<td>7</td>
<td>7</td>
<td>16</td>
<td>23</td>
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<tr>
<td>Severe</td>
<td>ND</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>22</td>
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<td>Months to maximal</td>
<td>ND</td>
<td>ND (&quot;acute&quot;)</td>
<td>5 (2–12)</td>
<td>ND</td>
<td>6</td>
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<tr>
<td>severity</td>
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<td></td>
</tr>
<tr>
<td>Pain</td>
<td>ND</td>
<td>7</td>
<td>4</td>
<td>9</td>
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<tr>
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<td>ND</td>
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<td>Cardiac involvement\textsuperscript{a}</td>
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<td>Overall response to treatment</td>
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<td>Poor</td>
<td>Variable: good,</td>
<td>3; partial, 3;</td>
<td>6; partial or</td>
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<td>2</td>
<td>3</td>
<td>0</td>
<td>4</td>
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<td>Serum creatine kinase</td>
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<td>ND</td>
<td>Mean: 12,946 IU/L (3064–25,000)</td>
<td>Median: 26.5 x N</td>
<td>mean: 6872 IU/L</td>
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ND, not determined.

\textsuperscript{a} Defined as evidence of cardiomyopathy or electrocardiographic evidence of arrhythmia or conduction abnormalities.

\textsuperscript{b} Evidenced by chest X-ray/computerized tomography scan.
Partial or complete resistance to steroids is a peculiar characteristic of anti-SRP myopathy. All our patients required the addition of methylprednisolone or IV Ig. Relapses are common during steroid tapering. Seven of 11 patients reported by Targoff et al. required cytotoxic agents and, in 4 of them, the disease failed to respond. Ten of 16 patients reported by Kao et al. were totally or partially resistant to steroids. Thirteen of 19 patients reported by Miller et al. were totally or partially resistant to steroid. Ten of 16 patients reported by Kao et al. required at least three drug trials and a need for combined immunosuppressive therapy (including methotrexate, azathioprine, cyclosporine, cyclophosphamide, and infliximab) or IV Ig was common. Interestingly, with such an aggressive regimen, favorable results were achieved in one third of patients. In our experience, addition of IV Ig was often efficient and safe. Moreover, aggressive combined treatment may be efficient, even if administered years after disease onset, as in patient 2 in this study. For patients resistant to common immunosuppressive therapy, however, plasmapheresis followed by rituximab was reported to be efficient in 2 patients.

Although clinical presentation of patients with anti-SRP myopathy usually suggests an acquired dysimmune origin, the misleading pathological features and apparent resistance to corticosteroid may delay diagnosis and treatment, thus worsening the prognosis. The most characteristic features of anti-SRP myopathy are: (1) rapid progression of weakness often culminating in severe disability; (2) markedly raised serum CK level; (3) necrotizing myopathy without inflammation or MHC-I immunostaining, and capillary pathology including MAC deposition; and (4) poor response to steroids. Early recognition of its stereotyped histopathological pattern should lead to a search for anti-SRP antibodies and, if these are present, to institution of immunomodulatory therapy. This work was supported by the Association Francaise contre les Myopathies (A.F.M.). The authors thank R. K. Gherardello and P. Cesaro for their helpful advice and contributions to this work.

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CASE OF THE MONTH

ABSTRACT: We describe a patient with Duchenne muscular dystrophy (DMD) who additionally suffered from intractable seizures, severe mental retardation, and a marked macroglossia. He also had endocrinologic abnormalities consisting of growth hormone deficiency, delayed puberty, and adrenal hypoplasia. We detected a duplication of DMD exon 18 and flanking introns that caused a frame-shift and was not removed by corrective splicing. A coincident mutation in the FKRP gene was excluded by direct sequencing. Complex DNA rearrangements, deletions, and duplications >100 kb were excluded through microarray–comparative genomic hybridization (CGH), although we were not able to exclude a second coincident mutation with certainty. In conclusion, we present a case of DMD that conflicts with current understanding of genotype–phenotype relations and discuss putative pathogenetic mechanisms for this uncommon phenotype.


TANDEM DUPLICATION OF DMD EXON 18 ASSOCIATED WITH EPILEPSY, MACROGLOSSIA, AND ENDOCRINOLOGIC ABNORMALITIES

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Duchenne muscular dystrophy (DMD) is caused by frame-shift deletions, duplications, or point mutations within the 79 exons of the dystrophin gene (DMD) on the X-chromosome.1,2 Depending on the location of the mutation, other organs besides the muscles, such as the central nervous system or the retina, may also be affected by the lack of dystrophin.20 At least seven different promoters drive the transcription of tissue-specific dystrophin isoforms that are named according to their molecular weight. The position of the mutation thus determines whether only the full-length dystrophin or also the truncated isoforms of the retina and central nervous system are affected.10,20 C-terminal mutations that involve the isoforms expressed in the retina (Dp260 or Dp71) predominantly result in abnormal electroretinograms (ERG) with reduction of the b-wave amplitude under scotopic conditions.18,12 Patients with C-terminal mutations that disrupt isoforms Dp140 or Dp71 often suffer from mental retardation.18,19

Here we describe a patient with a tandem duplication of exon 18 in the 5’ portion of the DMD gene, who—in addition to the predicted DMD phenotype—unexpectedly exhibited intractable epilepsy, marked mental retardation, absent b-waves in the ERG, macroglossia, short stature, delayed puberty, and adrenal hypoplasia.

CASE REPORT

Case History. The 19-year-old patient is the third son of healthy nonconsanguineous Caucasian par-
ents. Pregnancy and delivery were normal. During the neonatal period he had repeated apneas and his motor development was delayed. He was able to sit without assistance at 28 months but never learned to walk. At 2 years, progressive muscular atrophy with generalized muscular weakness, pseudohypertrophy of the calf muscles, and macroglossia (Fig. 1) became apparent. Serum creatine kinase levels were markedly elevated (9,000 U/L after birth and 600 U/L at 13 years; normal <269 U/L). The boy was mentally retarded and never learned to speak. Repeated cranial magnetic resonance imaging (at 6 and 12 years) demonstrated nonprogressive brain atrophy. In order to establish a diagnosis, we performed a muscle biopsy at 8 years. From that time, he also suffered from intractable epileptic seizures. Electroretinogram (ERG) at 10 years revealed absence of the b-wave under scotopic conditions, but nerve conduction velocities, and visual, auditory, and somatosensory evoked potentials were normal. At this time his macroglossia had to be reduced surgically, because it impeded proper swallowing. Despite the operation, however, his dysphagia necessitated artificial feeding through a gastrostomy from 11 years onwards.

Body length and weight were always below the third percentile (Fig. 1). At 10 years his bone age was delayed by 5 years and at 13 years by 3 years. Since the age of 15 years he has been artificially ventilated through a tracheostomy. Progressive scoliosis worsened his respiratory status further and at 17 years scoliosis surgery was tried but aborted intraoperatively because of severe osteopenia. Radiography showed extensive demineralization, which was caused neither by vitamin D deficiency (25-OH-cholecalciferol 42 nmol/L; normal 25–125; 1,25-OH-cholecalciferol 106 pmol/L; normal 70–220) nor by hyperparathyroidism (intact parathyroid hormone 15 pg/ml; normal 12–72; alkaline phosphatase 176 U/L; normal 130–700; normal urine excretion of calcium and phosphate).

**Endocrinologic Findings.** The patient’s testes did not descend until puberty and his adrenarche was delayed until 15 years of age. At 14 years, ultrasound and magnetic resonance imaging confirmed hypo-plastic, maldescended testes in the inguinal canal (0.4–0.5 ml each) and marked adrenal hypoplasia. His genitals were still infantile. At 19 years, the left testis had descended (3.5 ml, normal consistency), whereas the right testis was not palpable. Hormonal testing revealed growth hormone deficiency and confirmed delayed puberty (Table 1). There was evidence of functional testicular tissue [normal long human choriogonadotrophin (hCG) test], absence of ovarian tissue [negative human menopausal gonadotrophin (hMG) test], normal gluco- and mineralocorticoid production (normal tetracosactrin test and cortisol profile; normal serum levels of renin, aldosterone, and ACTH), normal thyroid function (normal thyroid stimulating hormone, triiodothyronine, thyroxine, and thyrotropin-releasing hormone test), and normal catecholamines.

**Immunohistochemistry and Western Blot.** Light microscopy revealed chronic dystrophic changes and increased fibrosis in the quadriceps and tongue muscle biopsy specimens. Immunostaining with monoclonal antibodies against the mid-domain (dys 1), the carboxy- (dys 2), and the amino-terminal (dys 3) domains of dystrophin confirmed its complete absence. Immunoreactivity for α- and β-dystroglycan, and α-, β-, γ-, and δ-sarcoglycan was markedly reduced; it was increased for utrophin. α2-, β1-, β2-, and γ1-laminins and spectrin 2 were normally expressed. All histologic examinations were performed as previously described.26 As expected from the immunohistology, we did not detect any residual or truncated dystrophin protein on Western blot with the above-mentioned antibodies.

**Chromosomal Studies and Array-CGH.** Conventional high-resolution karyotyping was normal. In order to look for a chromosomal rearrangement with multiple loci involvement and for genome-wide microdeletions that might have gone unnoticed by the karyotyping, we performed a whole-genome microarray-based comparative genomic hybridization (CGH). This was carried out using a 36k whole human genome tiling path BAC array consisting of the 1 Mb Sanger Clone set,5 a set of 390 subtelomeric clones, and the human 32k Re-Array set.17,23 The 36k array has a genome-wide resolution of ~100 kb. Hybridization and analysis with the software CGH-PRO was performed as described previously.3,14 However, besides three copy number changes located at 8q21.2 (loss), 5q31.3 (gain), and 18q21.1 (gain), which coincided with frequently found polymorphisms as listed in the Database of Genomic Variants (http://projects.tcag.ca/variation/), we did not find any abnormalities.

**Mutation Detection.** Southern blot was performed after restriction digestion with Hind III, Bgl I, Eco RI, and Pst I with various probes containing different segments of the DMD coding region as described previously.15 The probe specific for DMD exon 18 detected a larger fragment than in controls, which indicated a change of the molecular structure in this region (Fig. 2A). All other Southern blot results were normal. Polymerase chain reaction (PCR) analysis and subsequent automatic sequence
analysis revealed a tandem duplication of about 2.1 kb in DMD, which contained exon 18 and its flanking introns (Fig. 2B,D). Breakpoints were identified in introns 17 and 18 (Fig. 2D). The isolated duplication of DMD exon 18 was additionally verified through multiplex ligation-dependent probe amplification (MLPA) analysis of all 79 exons (MRC–Holland, Amsterdam, The Netherlands). We applied reverse transcription PCR (RT-PCR) and sequence analysis to verify the duplication of exon 18 on the level of the mRNA from the tongue (Fig. 2C–F).

Radioactive hybridization with an exon 15–17 as well as an exon 18 specific probe on the blotted exon 14–20 PCR products and subsequent analysis with a phosphor imager ruled out a corrective splicing mechanism (Fig. 2E). Additionally, we applied LightCycler (Roche, Basel, Switzerland) real-time RT-PCR for quantitative analysis of the transcripts from DMD exon 18. The Ct values of the products were corrected for different lengths and were normalized to the HPRT1 housekeeping gene. The marginally smaller amount of DMD exon 17–20 mRNA in the patient (cycle number ratio, mean \( \pm \) SEM, 0.67 \( \pm \) 0.01) than the three normal controls (0.79 \( \pm \) 0.03) excluded substantial nonsense-mediated messenger decay of the exon18dup allele. Because of the patient’s tongue hypertrophy, we also excluded a mutation in the fukutin-related protein (FKRP) gene by direct sequencing of its entire coding region.1

**DISCUSSION**

According to the reading frame-shift hypothesis, mutations that maintain the reading frame usually cause a semifunctional dystrophin and a milder phenotype, the Becker muscle dystrophy (BMD). In DMD patients, frame-shift mutations result in an unstable mRNA that is degraded by nonsense-mediated messenger decay (NMD), leading to a severe phenotype. The frame-shift hypothesis holds true in over

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**Table 1. Endocrinologic data**

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Normal range</th>
</tr>
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<tbody>
<tr>
<td>DHEAS (nmol/L)</td>
<td>&lt;128(^{†})</td>
<td>384–7168 at ( \geq ) 10 years and Tanner</td>
</tr>
<tr>
<td></td>
<td>497(^{‡})</td>
<td>2–3</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>2.8(^{§})</td>
<td>9.7–52.4</td>
</tr>
<tr>
<td>LH (U/l)</td>
<td>4.6(^{§})</td>
<td>0.8–7.6</td>
</tr>
<tr>
<td>FSH (U/l)</td>
<td>12.2(^{†})</td>
<td>1.5–14.0</td>
</tr>
</tbody>
</table>

**Gonadotropin-releasing hormone test**

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH maximal (U/L)</td>
<td>2.8(^{†})</td>
<td>0.9–7.0 at Tanner 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8–12.0 at Tanner 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5–15.0 at Tanner 3–4</td>
</tr>
<tr>
<td>FSH maximal (U/L)</td>
<td>3.6(^{†})</td>
<td>1.5–6.9 at Tanner 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2–5.5 at Tanner 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2–6.1 at Tanner 3–4</td>
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**Growth hormone axis**

**Growth factors**

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<th>Patient</th>
<th>Normal range</th>
</tr>
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<tbody>
<tr>
<td>IGF-1 (nmol/L)</td>
<td>3.8(^{†})</td>
<td>12.3–43.2</td>
</tr>
<tr>
<td></td>
<td>9.7(^{§})</td>
<td>19.7–70.3</td>
</tr>
<tr>
<td>IGF-BP3 (( \mu )g/ml)</td>
<td>1.10(^{†})</td>
<td>2.10–4.30</td>
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<tr>
<td></td>
<td>1.23(^{§})</td>
<td>1.36–2.71</td>
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</table>

**Arginine test**

<table>
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<th>Test</th>
<th>Patient</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>hGH maximal (ng/ml)</td>
<td>4.03(^{†})</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

**Insulin tolerance test**

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<th>Test</th>
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<th>Normal range</th>
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<td>hGH maximal (ng/ml)</td>
<td>23.94(^{†})</td>
<td>&gt;10.00</td>
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<td>hGH median (ng/ml)</td>
<td>2.91(^{†})</td>
<td>&gt;3.60</td>
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<tr>
<td>hGH profile</td>
<td>5.15(^{†})</td>
<td>8.8 ± 0.7</td>
</tr>
<tr>
<td>hGH peaks/night</td>
<td>2.0(^{†})</td>
<td></td>
</tr>
</tbody>
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DHEAS: dehydroepiandrosterone sulfate; LH: luteinizing hormone; FSH: follicle-stimulating hormone, IGF1: insulin-like growth factor 1; IGF-BP3: IGF-binding protein 3; hGH: human growth hormone.

*Age 10 years.

\(^{†}\)Age 11 years.

\(^{‡}\)Age 14 years and Tanner 1.

\(^{§}\)Age 19 years and Tanner 3 [the hormone levels can only be interpreted with respect to the patient’s sexual maturity, which is rated through the Tanner scale (Tanner JM. Growth at adolescence. Oxford, UK: Blackwell Scientific Publications, 1962)].
FIGURE 1. Severe macroglossia that was surgically reduced. Patient at the age of 5 and 19 years. Percentile chart for body length and weight. Permission to use photo was granted by the patient’s parents.

FIGURE 2. (A) Southern blot analysis of genomic DNA (Eco RI, lanes 1–5; Pst I, lanes 6–10) with a DMD exon 18 PCR probe. Wildtype Eco RI and Pst I fragments were expected to have a size of 11 kb and 5.4 kb. In the patient (lanes 2 and 7), we found junction fragments (jf), about 2.1 kb longer than the wt fragments. Male (lanes 1, 3, 4, 6, 8, 9) and female (lanes 5, 10) controls. (M) 100-bp size marker.

(B) PCR on genomic DNA with intronic primers flanking DMD exon 18. In the patient (lane 2), we found the expected 255-bp fragment and a further band of about 2.3 kb that was absent in controls (lanes 1 and 3). (M) 100-bp size marker.

(C) RT-PCR on mRNA from muscle with primers located on exons 17 and 20. The control mRNA (lane 2) contains the expected band at 486 bp, whereas the patient’s mRNA (lane 1) contains a band at about 610 bp; empty template PCR-control, lane 3. (M) 100-bp size marker

(D) Schematic presentation of the patient’s rearranged DMD gene on mRNA and on genomic DNA level (not drawn to scale). Breakpoints in introns 17 and 18 give rise to a tandem duplication of exon 18. The position of the breakpoint in intron 18 is depicted by a blue arrow and the breakpoint in intron 17 by a red arrow.

(E) Hybridization with an exon 15–17 specific probe on the blotted RT-PCR products spanning exons 14–20 shows a single band in our patient that was larger than the control band. The entire absence of a wildtype band in the patient rules out corrective splicing. Hybridization with an exon 18 probe yielded identical results.

(F) Sequence analysis of the RT-PCR products of Figure 2C.
90% of patients with dystrophinopathies. However, some patients with in-frame deletions do not produce any dystrophin and have a DMD phenotype, whereas some BMD patients compensate for a predicted frame-shift. Variations in the efficacy of NMD might account for the phenotypic variability even within a single family. In our patient, a duplication of exon 18 disrupted the reading frame and created a premature TGA termination codon after four nonoriginal amino acids. In accordance with the frame-shift hypothesis, the patient has a severe DMD phenotype. Immunohistologic data and Western blot analysis confirmed the complete absence of dystrophin. However, as mutant DMD mRNA was detectable at almost normal levels in the patient muscle, NMD does not seem to be responsible for the complete absence of dystrophin immunoreactivity. The reduction of dystrophin protein must thus have occurred further downstream, most probably due to the instability of the translation product and subsequent degradation through the proteasome. A similar effect has been reported in BMD patients whose dystrophin levels were severely reduced despite above normal DMD mRNA transcript numbers.

In the present case, the generally held genotype-phenotype hypothesis can only partially explain the patient’s phenotype, since the short isoforms with an alternative first exon 3’ to intron 18 should not be affected through the patient’s duplication. The exclusive alternative exons of the retinal isoforms Dp260 and Dp71 splice into exons 30 and 63. With intact retinal isoforms, b-wave formation and timing should be normal. In patients with an abnormal ERG, only 13% of the deletion-mutation sites are located 5’ to exon 30. Lack of the affected full-length isoform Dp427 that colocalizes with Dp260 at the outer plexiform layer would not result in ERG abnormalities. Therefore, the exon 18 duplication does not explain the abnormal ERG.

Likewise, the alternative exons of the C-terminal isoforms Dp140 and Dp71, which predominantly contribute to brain function, are spliced into exons 45 and 63 and should be unaffected by the exon 18 duplication. N-terminal mutations that only disrupt the full-length brain isoform Dp427B are generally not associated with severe mental retardation, whereas mental retardation has been described in single cases with loss of full-length dystrophin. Epilepsy is a rare feature of DMD and encountered in less than 2% of patients. Macroglossia is a common finding in dystrophin-deficient cats, but not in humans. Macroglossia and severe mental retardation were described in a single DMD patient with a mutation at the splice donor site of DMD intron 69, and macroglossia alone was reported in a patient with Klinefelter’s syndrome and BMD with a homozygous deletion of DMD exons 45–47. It has, however, never been described to our knowledge in N-terminal mutations. As the α-dystroglycan immunoreactivity was reduced in the patient’s muscle tissue, we additionally excluded a mutation in FKRP to rule out limb-girdle muscular dystrophy type 2I, which may also be associated with tongue hypertrophy in some cases.

Short stature with normal bone maturation and normal secretion of growth hormone is a common feature of DMD. A dystrophin-negative patient with normal intelligence, short stature, delayed bone age, growth hormone deficiency, hypogonadotropic hypogonadism, and normal adrenal function has been described before. Congenital adrenal hypoplasia and hypogonadotropic hypogonadism can occur as part of a contiguous deletion syndrome together with DMD and glycerol kinase deficiency. In these cases a deletion of >3 Mbp affects the DAX1, the GK, and the DMD gene. Such a deletion, however, could be excluded in our patient through high-resolution microarray-CGH.

According to current understanding of DMD splice variants, epilepsy, severe mental retardation, macroglossia, an abnormal ERG, and the endocrinologic abnormalities in our patient cannot satisfactorily be explained through the duplication of exon 18. We therefore hypothesize on putative pathogenetic mechanisms: (1) The duplicated parts of introns 17 and/or 18 might contain an unidentified regulatory element (e.g., a cryptic enhancer) that might effect the transcription efficiency or splicing of the downstream short DMD isoforms. Such enhancers can influence gene expression from a remote position through bending of the DNA. Since we did not have access to mRNA splice variants of affected tissues beyond the muscle, potential changes in splicing patterns and steady-state mRNA levels could not be evaluated. (2) The pattern of multiorgan involvement might be the effect of modifying genes in the patient’s genetic background. A strong influence of the genetic background on the severity of the disease phenotype has recently been demonstrated in congenic mouse models for limb-girdle dystrophy that carried identical null mutations in the γ-sarcoglycan gene. Finally, a coincident second syndrome cannot be excluded with certainty.

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REFERENCES


The Circuitry of the Human Spinal Cord. Its Role in Motor Control and Movement Disorders
by E. Pierrot-Deseilligny and D. Burke, 642 pp., ill., New York, Cambridge University Press, 2005, $190

This new book fills a previously vacant niche in providing an understanding of the physiology and organization of the human spinal motor system. Moreover, the scope of this textbook is enormous, ensuring that it will soon occupy a prominent place on many bookshelves and become essential reading for those engaged in motor systems research or seeking to study mechanisms of movement disorders.

The book is an essential reference tool and a timely contribution from two leading scientists whose combined contributions span decades and in many ways helped to define the field itself. The first chapter reviews the electrophysiological methodology of examining human reflex and spinal interneuronal systems, and provides many valuable technical insights, as the authors detail the limitations of the methodologies used in human spinal cord experimentation.

The bulk of the book addresses the electrophysiological evidence that has revealed the organization of human reflex systems as well as detailing the segmental and descending control of reflexes and their likely function in generating movement. Each chapter opens with a summary of background and rationale from data derived from animal experiments. What follows is an up-to-date synthesis of over a century's work. Throughout the text the authors are mindful of the state-dependent organization of spinal interneuronal systems: a phenomenon that has been well described, for example, over the past 15 years from locomotion experiments in the cat, but whose impact remains poorly appreciated by many in the general field of motor control. The reflexes and spinal pathways discussed include those from muscle primary and secondary spindles; Golgi tendon organs; recurrent inhibition from Renshaw cells; the flexion reflex pathways; cutaneousmuscular reflexes; the cervical and lumbar propriospinal system; and pre- and postsynaptic control from segmental and descending systems. Extrapolating heavily from animal data as necessary, the authors summarize how sensory feedback helps generate, sculpt, and execute movements such as standing, walking, reaching, and precision grip. The potential contribution of spinal reflexes to aberrant movement in disease states such as spasticity, Parkinson's disease, and other disorders is less well understood but a noble attempt is made to discuss these issues. The authors reinforce the evidence that convergence occurs between descending motor commands and sensory feedback on common neurons that then project to motoneurons. Readers will be left with a deeper appreciation of the blurred distinction between voluntary and reflex motor control, because both are inextricably and intimately linked to virtually all movement and posture. The frequent acknowledgment and discussion of the inherent technical limitations of electrophysiological studies in humans is enlightening and is a testament to the rigor and scrutiny that has characterized the scientific contributions of the authors.

No textbook is perfect. I was a little surprised there was no discussion of the important data on the group II reflex systems during locomotion in the cat from the Edmonton and Winnipeg groups. In addition, there was no mention of the aberrant control of the flexion reflex that has been described in the condition of periodic limb movements in the restless leg syndrome. Finally, stiff-person and stiff-leg syndrome—which are of presumed primary spinal origin—were also not addressed. These points, however, are trivial in comparison to the broad scope and authoritative-ness of this book. The figures selected are clean, elegant, and economic in their design and, thankfully, lack the needless flourishes that often confuse rather than clarify. Many of the figures are taken from original publications. Although some figures are complicated, none is more complicated than necessary.

With the exception of the chapter resumés, this textbook is not light reading, but the effort spent will offer lasting reward. The book will have broad appeal and application. Each chapter resumé is a useful stand-alone for those seeking the “big picture.” Within the chapters, however, sufficient detail is provided to refresh the memories and outlooks of even senior scientists engaged in human or animal spinal cord research. In the end, this textbook should be read thoroughly by all neurologists, neurophysiologists, or physiatrists interested in how the nervous system generates purposeful movement. I encourage these people to buy it, read it, and then re-read it!

Michael J. Angel, MD, PhD

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