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The Paradox of Muscle Hypertrophy in Muscular Dystrophy

Joe N. Kornegay, DVM, PhD[a,b,c,d,*], **Martin K. Childers, DO, PhD**[e], **Daniel J. Bogan, BA**[a,c,d], **Janet R. Bogan, BS**[a,c,d], **Peter Nghiem, DVM**[f], **Jiahui Wang, PhD**[g], **Zheng Fan, MD**[b,d], **James F. Howard Jr, MD**[b,d], **Scott J. Schatzberg, DVM, PhD**[h], **Jennifer L. Dow, BS**[a,c,d], **Robert W. Grange, PhD**[i], **Martin A. Styner, PhD**[g,j], **Eric P. Hoffman, PhD**[f], and **Kathryn R. Wagner, MD, PhD**[k,l]

[a]Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599, USA

[b]Department of Neurology, School of Medicine, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599, USA

[c]Gene Therapy Center, School of Medicine, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599, USA

[d]Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599, USA

[e]Section of PM&R, Department of Neurology, School of Medicine, Wake Forest University Health Sciences, Room 258, Dean Biomedical Research Building, 391 Technology Way, Winston-Salem, NC 27101, USA

[f]Department of Integrative Systems Biology, George Washington University School of Medicine and Research Center for Genetic Medicine, Children's National Medical Center, 111 Michigan Avenue, NW, Washington, DC 20010, USA

[g]Department of Psychiatry, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599, USA

[h]Veterinary Emergency and Specialty Center of Santa Fe, 2001 Vivigen Way, Santa Fe, NM 87505, USA

[i]Department of Human Nutrition, Foods, and Exercise, Virginia Tech University, 321 Wallace Hall, Blacksburg, VA 24061, USA

[j]Department of Computer Science, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599, USA

[k]Center for Genetic Muscle Disorders, Kennedy Krieger Institute, 801 North Broadway, Baltimore, MD 21205, USA

[l]Departments of Neurology and Neuroscience, The Johns Hopkins School of Medicine, 707 Broadway, Baltimore, MD 21205, USA

[*]Corresponding author. School of Medicine, Campus Box 7525, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599., joe_kornegay@med.unc.edu.

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> By the age of 3 years, his mother noted that his lower extremities grew in volume. Her attention was first drawn to this enlargement of his calves which entered his stockings with difficulty.
>
> —Duchenne's description of his first patient, Joseph Sarrazin, as cited by Tyler[1]

Mutations in the human dystrophin gene cause 2 clinical phenotypes, Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD), distinguished principally based on the age at which patients lose the ability to ambulate.[2,3] Boys with DMD become wheelchair users before 14 years of age, whereas those with BMD walk beyond age 16 years.[4] The basis for this clinical distinction can largely be traced to the fact that DMD mutations result in the loss of the mRNA reading frame, virtually eliminating dystrophin protein production, and BMD mutations preserve the reading frame, allowing production of a partially functional protein.[5] Muscles of patients with both forms express variable pathologic changes that generally lead to profound muscle atrophy. In contrast, some muscles, most notably the gastrocnemius, enlarge. Although hypertrophy hastypically been attributed to deposition of fat and connective tissue, so-called pseudohypertrophy,[6,7] imaging studies have shown true hypertrophy in some individuals.

There are 3 mammalian models in which spontaneous dystrophin gene mutations lead to distinct phenotypes of muscular dystrophy: the mdx mouse,[8,9] golden retriever muscular dystrophy (GRMD) dog,[10,11] and feline hypertrophic muscular dystrophy (FHMD) cat.[12,13] The GRMD model most closely mirrors DMD at multiple levels, including progressive disease that leads primarily to muscle atrophy. The mdx mouse progresses through an initial phase of muscle hypertrophy followed in old age by atrophy. In contrast, the FHMD cat has persistent muscle hypertrophy. The role of true hypertrophy has been more broadly accepted in these models, with less attention paid to contributions made by fat and connective tissue in muscle enlargement.

In principle, relative muscle sparing or hypertrophy could be either beneficial or detrimental. The benefits are obvious because preservation of strength should enhance motor function in activities ranging from ambulation to breathing. Harmful effects are less clear and relate to the potential for muscle hypertrophy to exacerbate contractures and postural instability. Additional deleterious consequences occur due to difficulties in eating because of glossal hypertrophy and regurgitation resulting from either esophageal hypomotility or obstruction at the level of the hypertrophied diaphragm. Whether the hypertrophy results from an actual increase in muscle mass or fat and connective tissue, studies directed at defining underlying mechanisms could provide insight into the pathogenesis of dystrophin deficiency and inform treatment development.

VARIABLE MUSCLE INVOLVEMENT AND HYPERTROPHY

Effects of dystrophin deficiency vary among species, individuals, and muscles. Reasons for phenotypic variation are poorly understood and raise questions about primary versus secondary effects of dystrophin deficiency.[14]

DMD and BMD

Extensor muscles that undergo eccentric muscle contraction, such as the quadriceps femoris, are particularly vulnerable in DMD.[15] In contrast, the extraocular muscles[16] are largely spared, and other muscles undergo striking paradoxical hypertrophy. Although attention has focused on gastrocnemius (calf) enlargement (Fig. 1), hypertrophy occurs in many other muscles. Presumptive cases of DMD characterized by hypertrophy of the calves, deltoid, and infraspinatus muscles were seen in Italy and England as early as the 1830s well before Duchenne's classic account.[1] In a 1995 review of 84 patients with DMD from India, 94% had calf enlargement, followed by the infraspinatus (88%), deltoid (52%), and tibialis anterior (40%).[17] The selective muscular involvement extended to different heads of the deltoid and quadriceps, which showed concomitant atrophy and hypertrophy. Calf hypertrophy was evident on physical examination in 20 of 26 (77%) BMD cases in another study.[18] These patients were further studied with computed tomography to determine the pattern and course of muscle involvement. Hypertrophy was seen in the calves (42%), sartorius (42%), gracilis (42%), adductor longus (38%), semitendinosus (19%), and rectus femoris (11%). Patients with DMD and/or BMD have also been shown to have hypertrophy of the tongue (macroglossia),[19] diaphragm,[20] and hypothenar (palm)[21] muscles, among others.

Dating to Duchenne's monograph, muscle hypertrophy in DMD and BMD has been attributed to deposition of fat and connective tissue, giving rise to the term pseudohypertrophic muscular paralysis.[1] Indeed, histopathologic studies have documented fibrosis and fatty change in the calves and other hypertrophied muscles.[6,7] Walton[22] speculated that true hypertrophy also contributes to muscle enlargement, perhaps occurring early in the disease course, followed by pseudohypertrophy. However, because of inherent limitations of sequential muscle sampling in human patients, the time course and relative roles of true hypertrophy and pseudohypertrophy have remained unsettled. Specialized imaging techniques have complemented and, in some cases, essentially replaced histopathologic evaluation of muscle biopsy samples. Increasingly, use of these techniques has documented features consistent with a true increase in contractile mass. In one such study, 10 of 16 patients with BMD had calf enlargement on ultrasonography, and 9 of them were judged to have true hypertrophy.[23] Another study, cited earlier, found that enlarged muscles visualized with computed tomography were often rounded and had normal densities, suggesting true hypertrophy.[18] A further paper, in which magnetic resonance imaging (MRI) was evaluated, showed that the gracilis and sartorius muscles were relatively spared and/or hypertrophied in 10 patients with DMD.[24] We are particularly intrigued by the relative sparing of the sartorius muscle (Fig. 2) because of our own studies of cranial sartorius hypertrophy in GRMD (see later).[25]

Ideally, imaging and histopathologic results should be correlated to define the relative contributions that fat, fibrous connective tissue, and myofiber hyperplasia or hypertrophy make to muscle enlargement. Few such studies have been completed in DMD. In one paper that correlated computed tomography and histochemistry findings, apparent true hypertrophy of the gastrocnemius in a 7-year-old boy with DMD was judged to be caused by myofiber hyperplasia rather than hypertrophy.[6] With this said, hypertrophied myofibers are also seen early in the disease and persist throughout life.[7]

The mdx Mouse

More extensive pathologic studies can be done with animal models, potentially allowing better definition of mechanisms contributing to differential muscle involvement. Just as in DMD, muscles are variably affected in the mdx mouse, ranging from the unaffected extraocular muscles[26] to the severely involved diaphragm.[27] Limb muscles undergo dramatic necrosis at 3 to 4 weeks of age followed by robust regeneration and muscle hypertrophy.[28–31] Changes vary among muscles, with the predominantly slow twitch soleus being more involved at 3 to 4 weeks[29,32,33] and the fast twitch extensor digitorum longus at 32 weeks and beyond.[29,33] Muscle hypertrophy causes mdx mice to be larger than normal between about 10 and 40 weeks of age, after which they lose weight in concert with a loss of muscle mass.[28,29,33] Reflecting the early strong regenerative response, mdx mice have increased numbers of small regenerating fibers with central nuclei at 3 weeks of age.[28,32,34] The percentage of larger regenerated myofibers increases with age, approaching 70% and 90% in the soleus and extensor digitorum longus, respectively, by 26 weeks.[32] Because populations of both small and large fibers occur concomitantly, mean fiber diameter of mdx mice typically is normal.[28,35] The concomitant occurrence of large and small fibers suggests that both hyperplasia and hypertrophy contribute to muscle hypertrophy.[31]

Unlike DMD, mdx mice have minimal fatty change and fibrosis,[33] indirectly implicating true hypertrophy as the cause for muscle enlargement.[31] The time course of pathologic lesions in the mdx mouse leads to corresponding functional changes, with affected mice being weaker at 2 to 4 weeks and then recovering.[36–38] Absolute tension generated by mdx muscles is typically higher by 8 to 16 weeks of age.[31,36,39] When corrected for cross-sectional area, isomeric soleus muscle force of younger (≤100 days) mdx mice was lower than normal, whereas values for older (≥100 days) mdx mice were higher.[39] Coulton and colleagues[39] commented that, “when mdx mice lose muscle fibers by necrosis, they do not just replace them with equally efficient new muscle, but with heavier, stronger, muscles than the wild-type.” Typical of this pattern and consistent with our own findings in the GRMD dog (see later), isometric tension generated by the tibialis anterior is considerably lower in mdx mice at 3 to 4 weeks[36] but exceeds normal values by 8 to 16 weeks.[31,36] Pathologic changes in the tibialis anterior also occur earlier than those in the soleus and gastrocnemius muscles.[36] Taken together, these mdx mouse data show clear evidence that early necrosis leads to a dramatic regenerative response that can lead to functional hypertrophy.

Contraction kinetics in mdx mice also differ from normal. Relaxation times were increased in the soleus, independent of age,[40] and in the tibialis anterior at 3 to 4 weeks[36] but not at later ages.[36,37] Although twitch-tetany ratios were decreased compared with normal in the

soleus of variably aged mdx mice,[40] a reverse relationship was seen in the tibialis anterior at 7 to 8 weeks.[37] Relaxation times of the mdx tibialis anterior were also increased at 3 to 4 weeks,[36] but values normalized in older mice.[36,37]

FHMD

Male cats with weakness, histologic features typical of muscular dystrophy, and gross and histologic evidence of muscle hypertrophy were characterized before the discovery of dystrophin.[41] Subsequently, an analogous syndrome was reported in cats with dystrophin deficiency.[12,13,42] One of these FHMD cats was shown to have a dystrophin gene deletion that included the skeletal muscle and neuronal Purkinje cell promoters and first exons.[42] The most striking clinical feature in all these cats was gross hypertrophy of their axial and appendicular muscles, as well as the tongue, diaphragm, and esophageal muscularis. In one study, 4 muscles (biceps brachii, cranial tibialis, gastrocnemius, and diaphragm) from 2 FHMD cats weighed twice as much as those from comparably sized normal cats.[12] Physical evidence of hypertrophy has been noted as early as 3 months[13] and seems to become more pronounced with age. As an example, the circumference of the neck in one cat increased from 28 to 33 cm between 14 and 25 months of age.[12] On microscopic examination, FHMD cats have myofiber size variation, with increased populations of both small regenerating and hypertrophied myofibers and an overall mean myofiber diameter in the normal range.[43] In keeping with progressive hypertrophy, the mean myofiber diameter of FHMD cats increased more so than that of normal cats between 3 to 4 and 6 to 9 months of age. In addition, there was myofiber necrosis and splitting and both gross and individual myofiber mineralization. The relative absence of fibrosis in FHMD cats up to 2 years of age suggests that the muscle enlargement is most likely because of true hypertrophy.[12]

GRMD Dog

As with DMD and the mdx mouse, the extraocular muscles are largely spared in GRMD.[44,45] Other muscles become involved as a function of age and usage. Muscles that are used heavily in utero and early in life, such as the tongue, diaphragm, and limb flexors, are acutely necrotic during the neonatal period.[44,45] Extensor muscles demonstrate a more delayed pattern of involvement, reflecting their greater use in weight bearing. As with the mdx mouse, muscles that undergo early necrosis may then regenerate and even hypertrophy. In one of the original GRMD dogs studied by our group, the hamstrings, tongue, diaphragmatic crura, and esophageal muscularis were enlarged.[10]

A further study in our laboratory showed that most GRMD pelvic limb muscles atrophy, whereas the caudal and cranial sartorius and popliteus hypertrophy.[25] Cranial sartorius muscle weights were corrected for body weight and endomysial space to determine true muscle weights (g/kg) in 3 GRMD age groups (4–10, 13–26, and 33–66 months) and grouped normal dogs (6–20 months) (Table 1). Corrected GRMD weights in the younger dogs were greater than those of normal dogs, indicating that the cranial sartorius undergoes initial true muscle hypertrophy. Values of both older groups were less than those of the younger dogs, suggesting that the cranial sartorius muscle atrophies over time, with an associated increase in the endomysial space because of deposition of fat and connective tissue.

Our studies of force/torque generated by individual and grouped GRMD muscles are in keeping with these pathologic data and findings from mdx mice. We initially evaluated tension generated by the peroneus longus muscle.[46] Absolute twitch tension and both muscle- and body-weight-corrected twitch tension in GRMD dogs were low compared with normal littermates at 3 months of age. Tetanic tension was affected similarly. However, although absolute values were still reduced at 6 months, twitch and tetanic tension corrected for either muscle or body weight was not statistically different (Fig. 3), suggesting that the peroneus longus recovers from an initial period of necrosis. Moving forward, we have primarily evaluated force/torque generated by tarsal joint flexors (including the cranial tibialis and peroneus longus) and extensors (including the gastrocnemius and superficial digital flexor that is analogous to the soleus).[47] For these measurements, the peroneal and tibial nerves are stimulated percutaneously so that the paw pulls (peroneal nerve, flexion) or pushes against (tibial nerve, extension) a lever interfaced with a force transducer. In our initial study, force values were measured at 3, 4.5, 6, and 12 months of age. While absolute and body-weight-corrected GRMD twitch and tetanic force values were lower than normal at all ages, tarsal flexion and extension were differentially affected (Fig. 4). Flexion values were especially low at 3 months, whereas extension was affected more at later ages.

We have used tetanic tarsal joint force measurements to evaluate effects of prednisone (2 mg/kg) given to GRMD dogs for a 4-month period beginning at 2 months of age.[48] Tarsal extension force increased in treated versus control GRMD dogs, whereas flexion paradoxically decreased. In light of our force studies, we assumed that the paradoxical decline in flexion occurred because prednisone attenuated early necrosis in muscles such as the peroneus longus and cranial tibialis that would have otherwise led to functional hypertrophy.

Contraction kinetics in GRMD dogs for both the peroneus longus[46] and grouped tarsal joint flexors and extensors[47] also differed from normal. Post-tetanic potentiation for the peroneus longus was more pronounced in GRMD versus normal dogs at both 3 and 6 months. Twitch contraction and relaxation times were dramatically prolonged, and there was concomitant sustained electrical activity at or before 6 months of age in some severely affected dogs. For the grouped muscles, the twitch-tetany ratio was generally lower, post-tetanic potentiation for flexion values was less marked, and extension relaxation and contraction times were longer. As discussed further later, prolonged relaxation times with underlying sustained electrical activity and more generalized complex repetitive discharges could play a role in muscle hypertrophy.

POSTURAL INSTABILITY AND CONTRACTURES

Comparative aspects of gait abnormalities, postural changes, and joint contractures in humans and animal models must be interpreted in light of fundamental differences in conformation, some of which arise because of quadrupedal versus bipedal locomotion. As an example, the line of the pelvis from the tuber ischium to the wing of the ilium extends in a vertical plane, perpendicular to the walking surface, in humans but is oriented more horizontally, largely parallel to the ground, in quadrupeds. Thus, changes in pelvic orientation innately differ among patients with DMD, mdx mice, and GRMD dogs. In some

cases, muscle weakness in DMD causes the posture to shift toward one normally assumed by quadrupeds and vice versa. The pelvis tilts anteriorly toward a more horizontal position to maintain postural stability in DMD[49] but shifts toward a vertical plane in GRMD dogs[50] (see later). Another factor relates to whether the leg/limb stance is plantigrade (humans and mice) or digitigrade (dogs).[51,52] Humans and mice walk on their phalanges, carpal, and tarsal bones, whereas dogs normally walk only on their distal and intermediate phalanges (digits). With neuromuscular diseases, in general, dogs become more plantigrade in the pelvic limbs,[53,54] adopting a stance more in keeping with that normally used by humans. A third stance, termed unguligrade, involves walking only on the tip of the distal digit.[52,55] The fact that horses have this posture explains use of the term equinus in DMD patients who toe walk.

DMD and BMD

Most DMD natural history studies include measurements of muscle strength, joint contractures, and timed function tests. Results from these tests are used to track disease progression and offer insight on clinical milestones, such as the loss of ambulation and the need for ventilatory support. Contracture and muscle strength scores generally correlate, deteriorate synchronously over time, and contribute mutually to postural instability.[56,57] As discussed later, unequal muscle weakness in DMD precipitates a vicious cycle that can lead to debilitating contractures and loss of ambulation (Fig. 5).[58]

Generally, contractures are caused by inactivity and restricted motion of the affected joint,[59,60] with a subsequent increase of collagen cross-links in periarticular connective tissue.[61] Major causes include forced joint immobilization to stabilize fractures,[60] spasticity associated with upper motor neuron lesions,[62] and primary neuromuscular diseases.[63] Joint contractures occur more commonly in DMD than other neuromuscular diseases[63] and have long been recognized as a major factor in disease morbidity. In one review that included 43 patients with DMD, the ankle (34/43), knee (29/43), hip (29/43), and elbow (28/43) were affected most frequently.[63] The ankle was also most commonly affected in BMD, occurring in 4 of 7 patients. Despite the prominent role that joint contractures play in these dystrophies, causative mechanisms are not fully understood. Underlying abnormal positioning presumably occurs because of an imbalance of forces acting on the joint, because diseased muscles are either disproportionately weakened or shortened by fibrosis. Relevant to this review, several studies have assessed the proportional strength of agonist and antagonist muscles operating at joints of patients with DMD. Some studies have concluded that muscular imbalance contributes to contractures,[57,64–66] whereas another found no such association.[67] Those finding a relationship noted a strong negative correlation between extensor muscle weakness and flexor contracture severity in DMD. As opposing extensor muscles weakened, flexor contractures worsened. Disproportionate flexor muscle hypertrophy would logically exaggerate this process. In a somewhat similar vein, contractures occurring because of spasticity also are magnified by enhanced flexor muscle activity.[62]

Postural instability and leg contractures are of special interest in DMD because of their role in the loss of ambulation. The relative sequence and proportional involvement of flexor and extensor muscles is critical. Early weakness of the hip (gluteus maximus) and knee

(quadriceps femoris) extensors necessitates postural changes to maintain ambulation. Increased anterior pelvic tilt and lumbar lordosis are adopted to shift the center of gravity forward of the knee and behind the hip, respectively.[49] Johnson[68] suggested that this posture allows passive ligamentous stabilization of these joints. Of comparative interest, a somewhat analogous system of ligamentous support (stay apparatus) allows horses to remain standing while sleeping.[69] Relative preservation of hip (including the sartorius) and knee (hamstrings) flexors in DMD creates destabilizing torque forces and also contributes to contractures at both levels.[70] Toe walking is adopted to stabilize the knee and later plays a role in the development of ankle equinus.[71] Plantar flexor contractures associated with equinus are aggravated by unbalanced muscle activity at the ankle, with selective weakening of the tibialis anterior and peroneus longus muscles and relative sparing of the triceps surae (collectively, the 2 heads of the gastrocnemius and soleus).[57,71] One investigator characterized this as a return to the infantile digitigrade pattern of walking, perhaps in an effort to put less stress on the weakened tibialis anterior muscle.[72] These contractures may initially have beneficial effects because tension in the gastrocnemius muscles pulls on the femoral condyles, extending the knee.[73] Iliotibial band (hip) contractures also extend the knee, providing additional stability. However, in advanced stages, heel cord and iliotibial band tightening destabilize gait, prompting the development of various corrective surgical procedures.[65,74] Scoliosis is typically a late complication of DMD, occurring in boys after they have gone into wheelchairs, with the side of convexity almost always toward the dominant hand.[75] The developing spine is thought to become deformed by excessive unbalanced forces from the dominant extremity. Scoliosis restricts expansion of the chest and tracks closely with respiratory disease.[76] Importantly, this biomechanical interplay in scoliosis shows that application of disproportionate (asymmetric) muscle force to the developing skeleton can cause bony maldevelopment with clinical consequences.

As discussed earlier, mechanisms contributing to preferential calf and other flexor muscle sparing or hypertrophy, starting with whether there is an actual increase in contractile tissue, are especially intriguing. If one accepts that these muscles undergo true hypertrophy in at least some patients, the timing and functionality of the muscle enlargement and whether it is playing a beneficial or detrimental role become critical. This would be particularly relevant with treatments, such as myostatin inhibition, that are intended to increase muscle mass (see later).

The mdx Mouse

Kyphosis analogous to scoliosis in DMD has been characterized in the mdx mouse. Just as with DMD, there is an association between spinal deformity and respiratory compromise. Mechanisms to account for kyphosis have not been defined, although Laws and Hoey[77] speculated that muscle hypertrophy could contribute. In a separate proof-of-concept study, this same group evaluated the potential for intramuscular injection of antisense oligonucleotides into paraspinal muscles to ameliorate kyphosis.[78] The treated group had less thoracic deformity than controls, but other outcome parameters did not differ between the groups. Weights of the latissimus dorsi, diaphragm, and intercostals muscles were increased in mdx mice in both treated and control mice. Force generation by these muscles was not increased, and histologic features were more in keeping with muscle degeneration

and fibrosis than true hypertrophy. Thus, although not the central focus of this study, there was not a definite link between muscle hypertrophy and kyphosis.

Contractures of the tarsal (talocrural, ankle) joint have recently been characterized in the mdx mouse.[79] As discussed earlier, both mice and humans have a plantigrade stance, so there could be analogous operative forces. In keeping with findings from DMD, mdx mice had plantar flexor contractures, evidenced by decreased dorsiflexion and range of motion. There was an associated increase in gastrocnemius wet muscle weight, and torque generated by the dorsiflexors was proportionally lower than that of the gastrocnemius muscles.

FHMD

Because of their muscle hypertrophy, FHMD cats have a stiff stilted gait with their hocks adducted.[12,13] They adopt a “falling down technique” to move into lateral recumbency and are unable to turn their heads and groom.[12] FHMD cats also do not close their mouths completely, presumably because of glossal hypertrophy,[12,13] which interferes with eating and swallowing, ultimately causing dehydration and azotemia.[13] The esophagus may have decreased contractility[13] and can be constricted by the hypertrophied diaphragm,[12,13] resulting in regurgitation. A syndrome similar to malignant hyperthermia has been characterized in several cats that died during stress or anesthesia (see discussion linking calcium homeostasis in this condition and muscle hypertrophy).[42,80]

GRMD Dog

Gait and postural changes in GRMD must be considered in the context of marked phenotypic variation among affected dogs.[81,82] Severely affected dogs may die within the first 10 days of life,[81] whereas others live well into adulthood.[83] By 6 weeks of age, GRMD dogs advance their pelvic limbs simultaneously (*bunny hopping*) and subsequently exhibit a progressively more stilted gait.[81] Those with a severe phenotype develop a characteristic plantigrade stance between 3 and 6 months, as evidenced by hyperextension of the carpus and hyperflexion of the tarsus.[81,84] Over this same period, the elbows become abducted and the hocks are adducted. Concomitantly, the pelvic limbs shift forward, as the tuber ischium of the pelvis moves ventrally and cranially. In severe cases, the line of the pelvis may be oriented in an essentially vertical plane, perpendicular to the walking surface (Fig. 6).[50] Some of these dogs lose the ability to walk and must be euthanized.

Beyond the initial 6 months of life, clinical signs in GRMD dogs tend to stabilize and further adaptive changes help to maintain postural stability. With both video gait analysis[85] and accelerometry,[86] GRMD dogs walk more slowly and their stride length is decreased. Accelerometry demonstrated a redistribution of power at gait, with a decrease in the craniocaudal plane and a compensatory increase mediolaterally to maintain balance.[86] On video gait analysis, older, generally mildly affected dogs had a more upright stance, with relatively greater extension of the stifle and lesser flexion of the tarsus.[85] This posture presumably is adopted in an effort to stabilize their stance in the face of quadriceps weakness. In our experience, GRMD dogs rarely become nonambulatory after the initial critical period of destabilization at 3 to 6 months of age. Some have a normal life span; we currently have a 12-year-old GRMD dog with a remarkably mild phenotype in our colony.

One of our earlier studies showed that 6-month-old GRMD dogs positioned in dorsal recumbency for force measurements have abnormally acute (contracted) tarsal joint angles.[46,84] Other investigators have subsequently described methods to measure joint angles at maximal flexion and extension in normal dogs.[87,88] We now use the method suggested by Jaegger and colleagues[87] to measure pelvic limb joint angles and range of motion. By 6 months of age, GRMD dogs tend to have more restricted maximal flexion of the hip joint, increased maximal stifle extension, and more acute maximal tarsal flexion. To objectively characterize the cranioventral shift of the pelvis, we also measure the angle formed by 2 lines extending cranially from the tuber ischium, one drawn parallel to the lumbar spine and the other extending to the midpoint of the tuber coxae. This angle is larger in dogs with GRMD than in normal dogs at 6 months of age.

Mechanisms contributing to postural and joint angle changes in GRMD dogs have not been defined. The cranioventral pelvic shift may be an adaptive response, as affected dogs move their pelvic limbs under the torso to maintain balance. The resultant posture is similar to that achieved by boys with DMD when they shift their pelvis forward.[49] Alternatively, unbalanced hip flexor and extensor strength might play a role. Relative preservation of the hamstring muscles in GRMD dogs could pull the tuber ischium ventrally and also contribute to decreased maximal hip flexion values. As discussed earlier, considering the role that the sartorius and iliotibial band play in hip flexor contractures in DMD,[74] true hypertrophy of the cranial sartorius could be playing an analogous role in GRMD. In support of a relationship, we have previously shown that cranial sartorius circumference correlates negatively with tarsal joint angle in affected dogs.[25] Still, it is unclear whether there is truly a cause-and-effect relationship. The hypertrophied cranial sartorius could actively pull the stifle joint forward, with the tarsus passively following to assume a plantigrade position. On the other hand, cranial sartorius hypertrophy and plantigrade stance might have common root causes but no direct functional relationship. In any case, cranial sartorius hypertrophy or contracture does seem to affect the developing pelvis in young GRMD dogs, as the ilial wings from which it originates flare laterally (see Fig. 6),[50] presumably in response to unopposed torque. This is somewhat analogous to scoliosis resulting from unbalanced force applied by the dominant arm in boys with DMD[75] and emphasizes the potential for disproportionate muscle size and strength to cause skeletal deformity.

Local imbalance of agonist and antagonist muscles could also be playing a role in postural changes at the tarsal and carpal joints of GRMD dogs. Consistent with findings in DMD, we have shown that GRMD extensor and flexor muscles operating at the tarsal joint are differentially affected. Flexion values are especially low at 3 months, whereas extension is affected more at later ages (see Fig. 4).[47] At 6 months of age, the tarsal extension-flexion force ratio correlates positively with tarsal joint angle, which is to say that dogs with stronger extensors have larger joint angles and a less severe phenotype.[89] We have not systematically studied joint angles in the thoracic limb and can only speculate on mechanisms involved in carpal hyperextension. It seems unlikely that a reverse pattern of muscle involvement, whereby extensor muscles are relatively preserved, is responsible. Importantly, plantigrade stance is a nonspecific clinical sign in dogs and cats with neuromuscular disease,[53,54] independent of underlying cranial sartorius hypertrophy. This suggests that the pelvic limb plantigrade posture, coupled with hyperextension of the carpus

(so-called palmigrade stance),[53] may simply reflect distal muscle weakness. The pivot point would logically differ between the carpus (hyperextension) and tarsus (hyperflexion) to keep the footpads in contact with the walking surface.

Spinal curvature is not a major feature of GRMD. Lumbar kyphosis may occur in tandem with the cranioventral shift of the pelvis; lordosis can be seen more chronically.[81] The lordotic posture could occur because of unequal application of force on the developing spine, with relative restriction of vertebral growth dorsally (posteriorly) or exaggerated growth ventrally (anteriorly).[90,91] Relative preservation of the psoas major muscle in GRMD dogs[25] might exert disproportionate force on the ventral lumbar spine, contributing to both hip flexor contractures and lordosis.

MECHANISMS CONTRIBUTING TO MUSCLE HYPERTROPHY

Differences in the severity, distribution, and nature of pathologic lesions leading to muscle enlargement in DMD and the 3 animal models should offer clues on mechanisms contributing to muscle hypertrophy in dystrophin deficiency. In the mdx mouse and GRMD cranial sartorius, muscle hypertrophy follows early necrosis. We believe that a convergence of factors driving muscle regeneration and differentiation leads to a disordered or overly robust proliferative response in GRMD. Although it is tempting to speculate on a similar mechanism in DMD and FHMD, no direct evidence links early necrosis and hypertrophy in these diseases. The relative lack of fatty infiltration in muscles of the mammalian models raises additional mechanistic questions relating to the efficiency of muscle regeneration among species and their propensity for fatty change secondary to muscle injury. These questions are generally beyond the scope of this article. Here, we review central concepts and suggest potential drivers for muscle hypertrophy in light of findings in affected animals.

Muscle mass can expand through either increased cell number (hyperplasia) or size (hypertrophy), with proliferation playing a greater role in developing muscle[92] and hypertrophy being more important in maturity.[93] Both myofiber hyperplasia and hypertrophy are thought to contribute to increased muscle size in DMD and the animal models.[6,7,25,31,43] Muscle size is regulated by a complex set of genes that establish a balance between forces that would otherwise lead to either atrophy or hypertrophy.[94] With regard to muscle mass in DMD, much attention has focused on insulin-like growth factor 1, which is known to promote muscle hypertrophy by activating the phosphatidylinositol 3 kinase/Akt pathway and, in turn, mTOR and further downstream targets.[95] Activation of the Akt-mTOR pathway is associated with muscle hypertrophy in the mdx mouse, and Akt expression is increased in DMD muscles.[96] These findings led the investigators to conclude that “vigorous activation of hypertrophic processes during prenecrotic stages of disease might reduce the severity of pathology or extend the prenecrotic stage of disease.”[96]

Many other factors could be driving or supporting muscle hypertrophy. Anderson and colleagues[97] found increased binding activity of basic fibroblast growth factor (bFGF) in mdx mouse versus DMD and GRMD muscle, suggesting that its expression might augment the regenerative response by recruiting more muscle precursor cells. However, in a subsequent study, administration of bFGF did not enhance muscle regeneration in mdx mice.

[98] Transforming growth factor β_2 (TGF-β_2) levels are increased in both newly formed myotubes of regenerating muscle[99] and in muscle samples from patients with DMD.[100] The glycoprotein osteopontin (OPN; secreted phosphoprotein 1) functions as a proinflammatory/fibrotic cytokine in mdx mice[101] and serves as a genetic modifier in DMD.[102] Importantly, OPN also promotes myogenesis[103] and so could play a dual role in transitioning muscle from an inflamed to regenerative state. We have found increased levels of both TGF-β_2 and OPN in GRMD muscles; moreover, TGF-β_2 tends to track positively with the degree of cranial sartorius hypertrophy (Nghiem and colleagues, unpublished data, 2011).

The complex repetitive discharges seen on electromyography in the 3 dystrophin-deficient animal models[10,13,104] and, to a lesser extent, in DMD[105] offer an additional mechanism to account for muscle hypertrophy. Sustained electrical activity (myotonia) and muscle contractions associated with chloride and sodium channelopathies can lead to dramatic muscle hypertrophy in the nondystrophic myotonias.[106,107] Spontaneous discharges in dystrophin-deficient muscle may occur because of altered ion currents across the damaged muscle cell membrane, with an associated shift in the resting membrane potential. Sustained muscle contraction would presumably augment muscle size, just as it does in myotonia. A positive correlation was observed between Akt pathway activity and muscle hypertrophy in a group of patients with myotonic dystrophy.[108]

While correlative data are largely circumstantial, abnormal calcium homeostasis could predispose to both muscle hypertrophy and susceptibility to malignant hyperthermia–like syndromes. Myofiber calcium levels are increased in DMD[109] and each of the 3 mammalian models.[13,110,111] Increased cytosolic levels of calcium also occur in malignant hyperthermia caused by mutations in the ryanodine receptor gene.[112] Myofiber hypertrophy is a predictor of susceptibility to malignant hyperthermia in humans[113] and occurs in susceptible dogs.[114] Although patients with DMD are not at increased risk for malignant hyperthermia itself, they do occasionally develop a similar syndrome when anesthetized with volatile gases.[115] Among the dystrophin-deficient animal models, cats are particularly prone to a malignant hyperthermia–like syndrome[80] and demonstrate the most striking muscle hypertrophy.[12,13] We have seen a similar metabolic syndrome in GRMD dogs anesthetized with isoflurane and sevoflurane. In addition, changes in contraction kinetics of the GRMD peroneus longus muscle occur as it is increasing in size and are compatible with those seen with a delay in calcium sequestration.[46]

MYOSTATIN INHIBITION IN MUSCLE DISEASE

Beyond the incalculable emotional trauma for patients with DMD and their families, society pays a huge price. Yearly health care costs exceed $30,000 per patient with DMD, 10 to 20 times those of other hospitalized groups.[116] In Australia, overall annual costs for each patient, including personal care and lost productivity, have been estimated to be $126,000 and yearly costs due to muscular dystrophy have been placed at approximately $1.5 billion.[117] These figures offer a window into the broader societal impact of more common degenerative muscle disorders such as sarcopenia and cancer cachexia. Sarcopenia incidence increases over life, with approximately 15% of women and 30% of men affected at age 80

years.[118] Direct health care costs attributable to sarcopenia in the United States approached $20 billion in 2000.[119]

The collective incidence and cost of health care for the muscular dystrophies and other muscle wasting disorders is a major driver for the pharmaceutical industry.[120,121] One strategy for promoting muscle regeneration involves inhibiting myostatin (*Mstn*; growth/differentiation factor 8), a negative regulator of muscle growth. Humans,[122] cattle,[123] sheep,[124] and dogs[125] with myostatin mutations have dramatic muscle hypertrophy. Dystrophin-deficient mdx mice in which myostatin is knocked out ($Mstn^{-/-}$)[126] or inhibited postnatally[127] also have a less severe phenotype. Conversely, results from other dystrophic murine models in which myostatin was knocked out have varied,[128,129] with some mice showing increased morbidity.[130] There are questions about potential senescence of myostatin-deficient cells that have undergone multiple divisions.[129] Moreover, abnormalities have been identified in muscle tendons from $Mstn^{-/-}$ mice.[131]

Although genetically engineered mice have provided an extremely powerful tool to study the molecular pathogenesis of disease, results do not necessarily extrapolate to humans, presumably because of differences between murine and human size and physiology.[132] These shortcomings are partially obviated with canine models, which have been used extensively to study disease pathogenesis and treatment efficacy.[133,134] This trend toward the use of canine genetic models such as GRMD to study human disease will likely accelerate with the recent sequencing of the canine genome.[135] Thus, ideally, results from myostatin-deficient mdx mice should be corroborated in dystrophic dogs in which the gene has been knocked out. Unfortunately, transgenic technology in the dog is very cumbersome and inefficient, essentially precluding use of this approach.[136] With this in mind, we have developed a canine (*GRippet*) model by crossbreeding dystrophin-deficient GRMD dogs with myostatin-heterozygous ($Mstn^{+/-}$) whippets.

A total of 4 dystrophic $Mstn^{+/-}$ *GRippets* and 3 dystrophic *Mstn* wild-type ($Mstn^{+/+}$) dogs from 2 litters have been evaluated using various phenotypic tests at 6 to 8 months of age. Rather than showing improvement, the dystrophic $Mstn^{+/-}$ *GRippet*s were more clinically affected than their dystrophic $Mstn^{+/+}$ littermates, apparently because of disproportionate enlargement of certain muscles. In particular, unequal hypertrophy of proximal pelvic limb muscles, as determined by MRI (Fig. 7 and Table 2) and necropsy, seemed to exaggerate postural abnormalities. Gross cranial sartorius muscle hypertrophy was particularly prominent. In general, atrophy or hypertrophy of individual muscles in dystrophic $Mstn^{+/+}$ dogs was exaggerated in the dystrophic $Mstn^{+/-}$ *GRippets*. Some dystrophic $Mstn^{+/-}$ *GRippet*s had commensurate, more pronounced atrophy/hypoplasia of the quadriceps femoris muscle.

Data from these *GRippet* dogs indicate that mechanisms contributing to selective muscle hypertrophy or atrophy/hypoplasia in dystrophin-deficient muscle may be exaggerated by partial loss of myostatin. Clearly, findings from a genetic model in which myostatin is inhibited from conception will not necessarily predict the outcome of staged postnatal treatments. However, at the very least, our results imply that differential effects of myostatin

inhibition on muscle could have deleterious consequences, in keeping with various other findings reviewed in this article.

SUMMARY

Mutations in the dystrophin gene cause Duchenne and Becker muscular dystrophy in humans and syndromes in mice (mdx mouse), dogs (golden retriever muscular dystrophy and other canine mutations), and cats (feline hypertrophic muscular dystrophy). While affected humans and dogs have progressive disease that leads primarily to muscle atrophy, certain muscles undergo paradoxical hypertrophy. Mdx mice progress through an initial phase of muscle hypertrophy followed by atrophy. Cats have persistent muscle hypertrophy. In both humans and animals, muscles are not uniformly affected, with some atrophying as others enlarge. Disproportionate involvement of flexor and extensor muscles acting at joints can exaggerate contractures. Muscle hypertrophy in humans has generally been attributed to deposition of fat and connective tissue (pseudohypertrophy), but recent imaging studies suggest that increased muscle mass (true hypertrophy) also occurs. True hypertrophy plays a more prominent role than fibrosis and fatty deposition in the animal models. Various mechanisms could potentially account for increased muscle mass and, thereby, be manipulated therapeutically. However, for maximal benefit, treatments should uniformly increase mass or preferentially improve strength of clinically-important muscles. Treatments that result in disproportionate muscle enlargement may exaggerate postural instability and joint contractures. These deleterious consequences of muscle hypertrophy should be considered when developing treatments for muscular dystrophy and other muscle wasting disorders.

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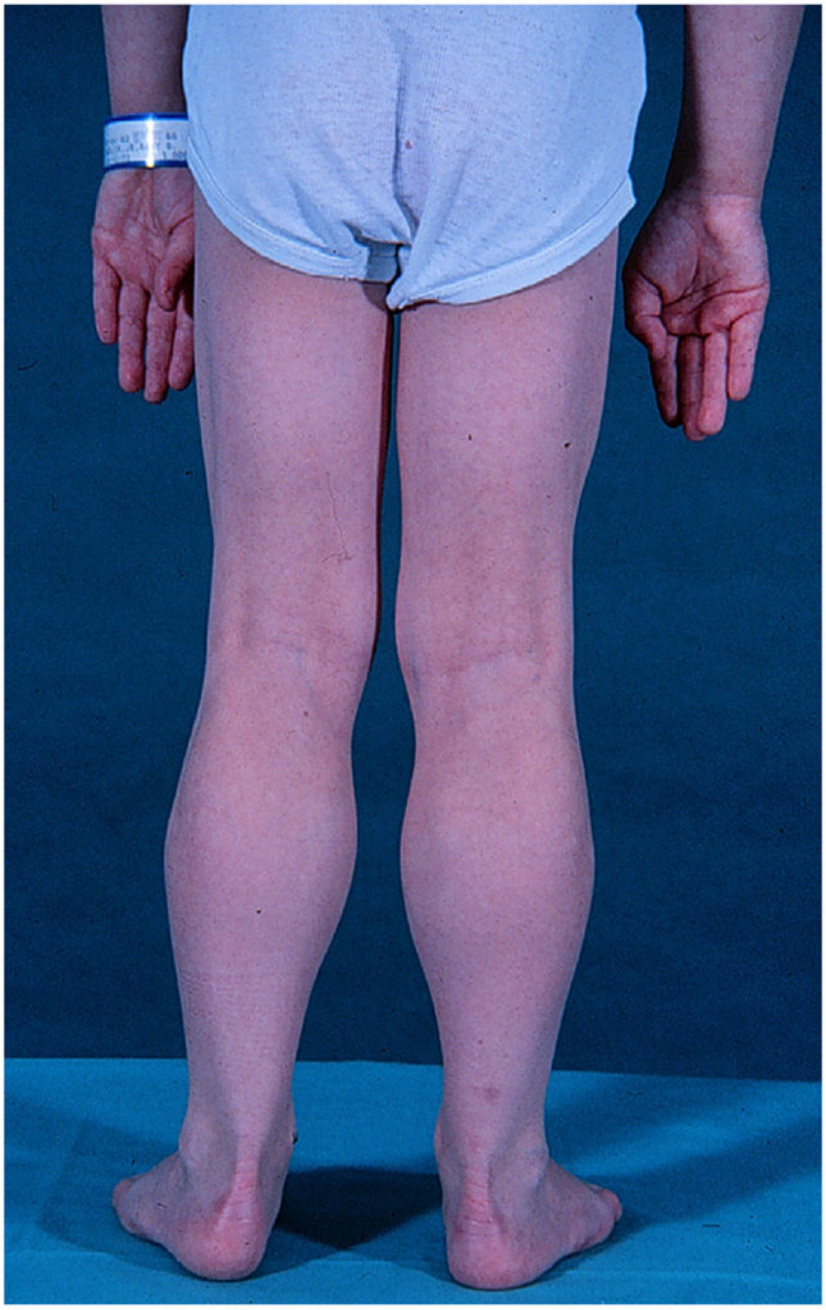

Fig. 1.
Nine-year-old boy with DMD demonstrating characteristic calf hypertrophy. (*Courtesy of* James F. Howard Jr.)

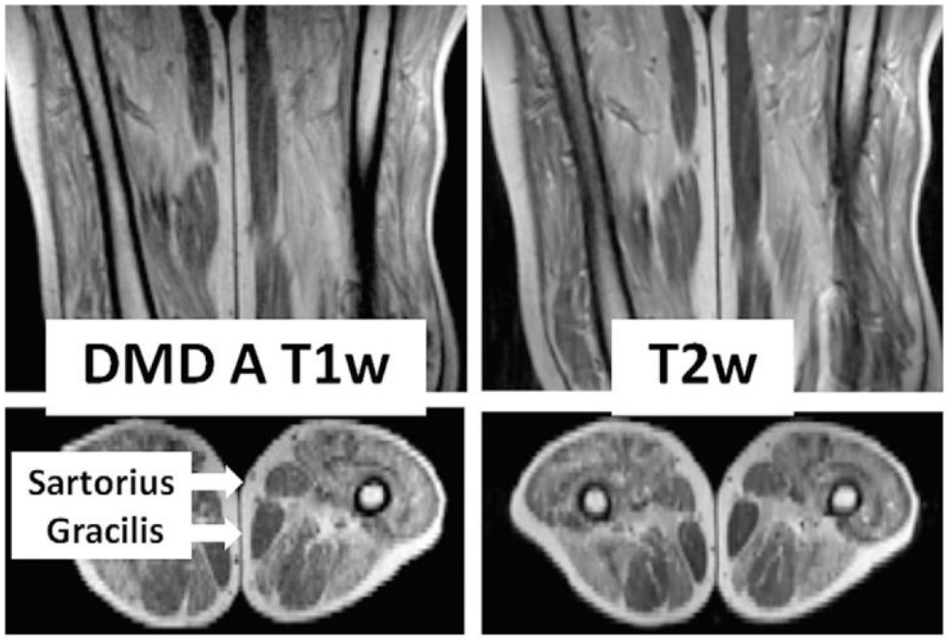

Fig. 2.

T1- and T2-weighted magnetic resonance images of the proximal leg of a boy with DMD in the anteroposterior (*top*) and transverse (*bottom*) planes. The transverse image is at the level of the midfemur. Most muscles have been partially to near totally replaced with signal-intense material compatible with fat. Note that the subcutaneous fat has comparable signal intensity in both T1- and T2-weighted images. In contrast to the properties of most muscles seen, the sartorius and gracilis muscles (*arrows*) are largely unaffected.

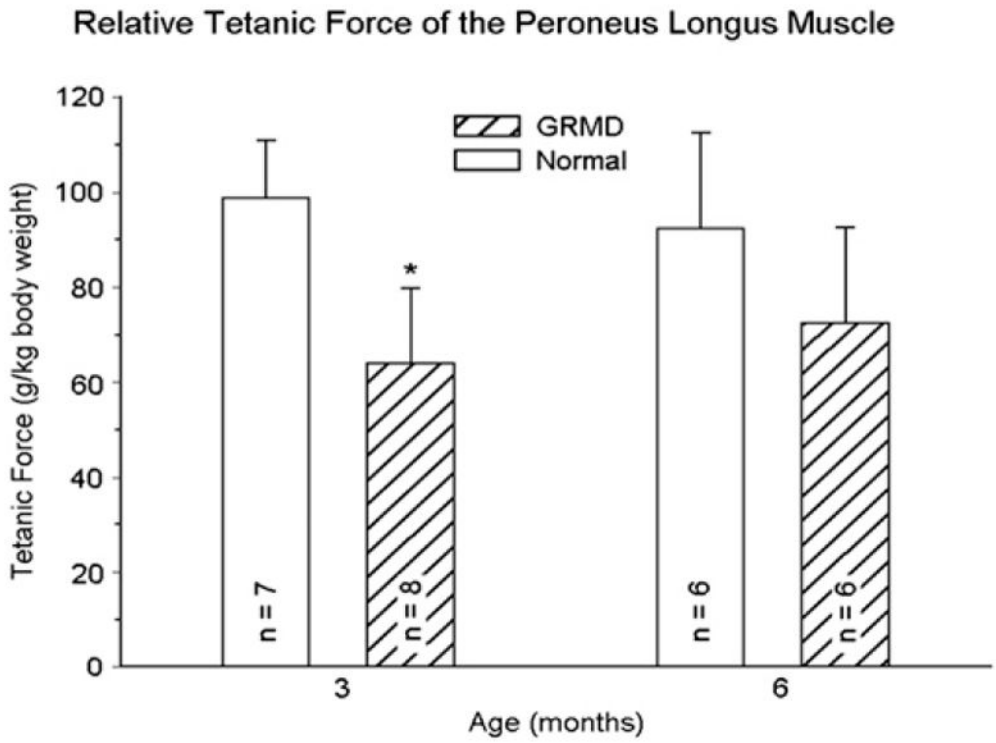

Fig. 3.

Tetanic force corrected for body weight (g/kg) generated by the peroneus longus muscle of normal and GRMD dogs at 3 and 6 months of age. Values for the GRMD dogs are lower (P<.05)* at 3 months. However, although body-weight-corrected force is proportionally lower in normal dogs at 6 months, it has increased in GRMD dogs, such that values for the 2 groups are no longer significantly different. (*Data from* Kornegay JN, Sharp NJ, Bogan DJ, et al. Contraction tension and kinetics of the peroneus longus muscle in golden retriever muscular dystrophy. J Neurol Sci 1994;123:100–7.)

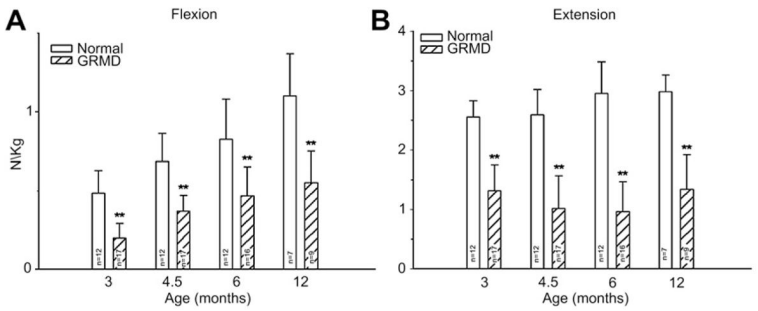

Fig. 4.

Tetanic force, corrected for body weight (N/kg), generated by tarsal joint flexors (*left*) and extensors (*right*) from normal dogs and GRMD dogs at 3, 4.5, 6, and 12 months of age. Values for GRMD dogs are lower ($P<.01$ for all) than those of normal dogs at all ages. However, the differential between GRMD and normal dogs differs. Flexion values are especially low at 3 months, whereas extension is affected more at later ages. (*From* Kornegay JN, Bogan DJ, Bogan JR, et al. Contraction force generated by tibiotarsal joint flexion and extension in dogs with golden retriever muscular dystrophy. J Neurol Sci 1999;166:119; with permission.)

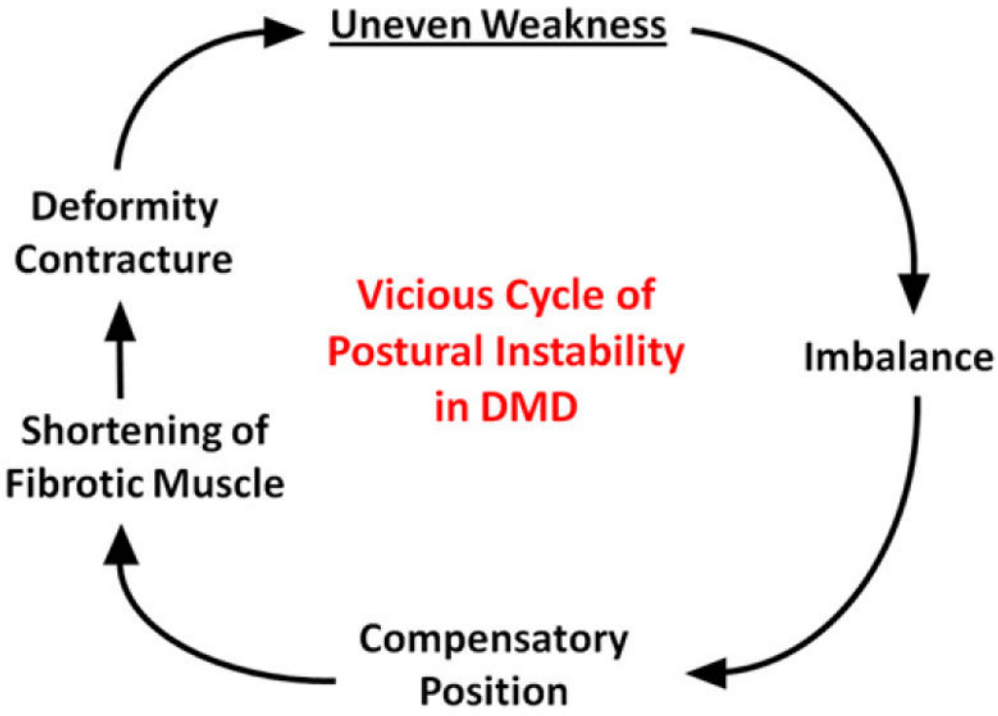

Fig. 5.

Vicious cycle of postural instability that leads to loss of ambulation in patients with DMD. Uneven weakness leads to imbalance and compensatory postural changes that ultimately result in shortening of muscles and contractures. The process is self-perpetuating. (*Modified from* Roy L, Gibson DA. Pseudohypertrophic muscular dystrophy and its surgical management: review of 30 patients. Can J Surg 1970;13:14; with permission.)

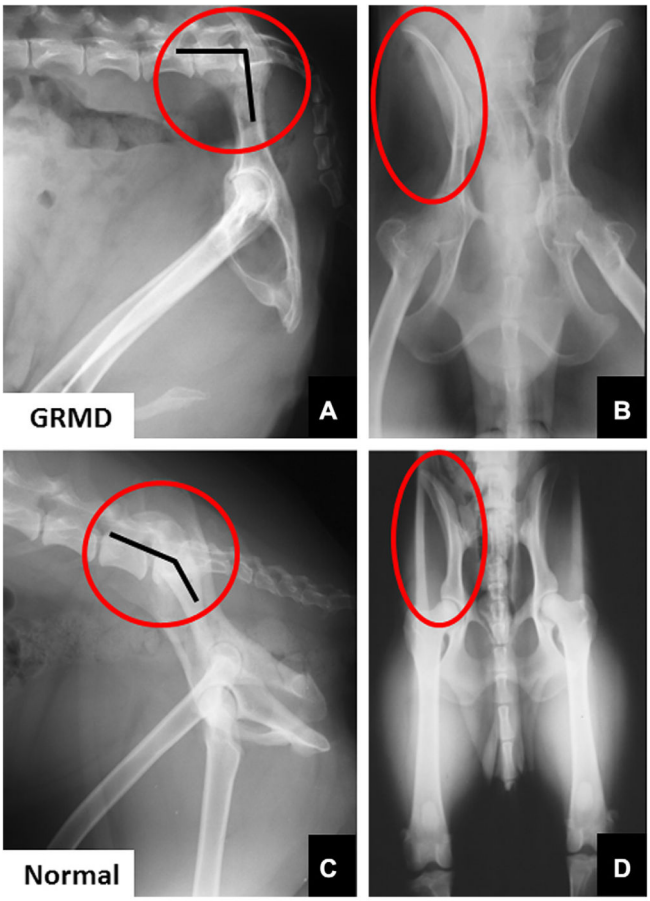

Fig. 6.

Lateral (*A*) and ventrodorsal (*B*) radiographs of the pelvis of a 4-year-old GRMD dog and a normal dog (*C* and *D*) for comparison. Note the marked vertical tilt of the pelvis in the GRMD dog (*A*), so that the angle formed by the wing of the ilium and lumbar spine is much more acute at approximately 90° (angle marked by lines within the red circle). The wings of the ilia flare laterally in the GRMD dog (*red circle* in *B*). (*From* Brumitt JW, Essman SC, Kornegay JN, et al. Radiographic features of Golden Retriever muscular dystrophy. Vet Radiol Ultrasound 2006;47:578; with permission.)

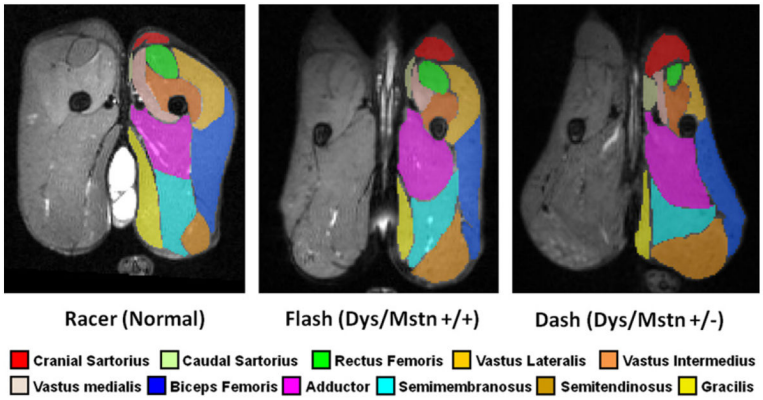

Fig. 7.

T2-weighted magnetic resonance images with fat signal suppression (FS) of pelvic limb muscles at midthigh from 3 crossbred GRMD and myostatin-heterozygous (*Mstn*$^{+/-}$) dogs from the first litter. Note the proportional enlargement of the sartorius and hamstring muscles and the associated atrophy/hypoplasia of the quadriceps of the dystrophic *Mstn*$^{+/+}$ dog, *Flash*, relative to the normal dog, *Racer*, and the even more dramatic differential size of these muscles in the dystrophic *Mstn*$^{+/-}$ dog, *Dash* (also see volumetric measurements from these sections in Table 2). Segmentation was done using ITK-SNAP (http://www.itksnap.org/pmwiki/pmwiki.php).[137]

Table 1

Morphometric GRMD vs. normal canine cranial sartorius histopathologic data (mean ± SD)

		GRMD		
		Group 1	**Group 2**	**Group 3**
Pathologic Lesion	**Normal (6–20 mo; n = 12)**	**4–10 mo; n = 15**	**13–26 mo; n = 4**	**33–66 mo; n = 4**
Corrected muscle weight (g/kg)[a]	1.3075 ± 0.2079	3.0573 ± 0.7635	2.4725 ± 0.7556	1.4650 ± 0.6575
Percentage of endomysial space[b]	2.8083 ± 1.3468	27.1857 ± 15.3869	44.1275 ± 14.6462	58.9100 ± 14.2060
True muscle weight (g/kg)[c]	1.2699 ± 0.1966	2.2063 ± 0.6884	1.3758 ± 0.5078	0.5720 ± 0.2423
Mean fiber diameter (μm)[d]	42.1658 ± 4.3542	57.4980 ± 11.7419	63.1295 ± 13.3033	47.0850 ± 17.7029

[a] Normal<GRMD, Group 1 (P<.05); GRMD Group 1>GRMD Group 3 (P<.05).

[b] Normal<GRMD Groups 1–3 (all P<.05).

[c] Normal<GRMD, Group 1 (P<.001); GRMD Group 1>GRMD Groups 2 (P<.05) and 3 (P<.001).

[d] Normal<GRMD Groups 1 and 2; P<.01 for Group 1 and P<.05 for Group 2.

Data from Kornegay JN, Cundiff DD, Bogan DJ, et al. The cranial sartorius muscle undergoes true hypertrophy in dogs with golden retriever muscular dystrophy. Neuromuscul Disord 2003;13:493–500.

Table 2

MRI volumetric measurements of proximal pelvic limb muscles in crossbred GRMD and Mstn$^{+/-}$ dogs[a]

	Racer (Normal/*Mstn*$^{+/+}$)			Flash (Dys/*Mstn*$^{+/+}$)			Dash (Dys/*Mstn*$^{+/-}$)		
Muscle	**Volume (mm^3)**	**Volume (mm^3/kg)**	**Percent of Section**	**Volume (mm^3)**	**Volume (mm^3/kg)**	**Percent of Section**	**Volume (mm^3)**	**Volume (mm^3/kg)**	**Percent of Section**
Cranial sartorius	101	5.87	1.77	168	16.15	4.25	309	30.00	8.01
Caudal sartorius	29	1.69	0.51	52	5.0	1.32	109	10.78	2.83
Sartorius total	***130***	***7.56***	***2.28***	***220***	***21.15***	***5.57***	***418***	***40.78***	***10.84***
Rectus femoris	279	16.22	4.89	181	17.40	4.58	69	6.70	1.79
Vastus lateralis	635	36.91	11.13	419	40.29	10.60	232	22.52	6.01
Vastus medialis	351	20.41	6.16	127	12.21	3.21	132	12.82	3.42
Vastus intermedius	480	27.91	8.42	207	19.90	5.23	130	12.62	3.37
Quadriceps total	***1745***	***101.45***	***30.60***	***934***	***89.80***	***23.62***	***563***	***54.66***	***14.59***
Semimembranosus	807	46.92	14.16	578	55.58	14.62	807	78.35	20.92
Semitendinosus	284	16.51	4.98	509	48.94	12.88	680	66.02	17.63
Hamstring total	***1091***	***63.43***	***19.14***	***1087***	***104.52***	***27.50***	***1487***	***144.37***	***38.55***
Biceps femoris	1156	67.21	20.28	643	61.83	16.27	612	59.42	15.86
Gracilis	634	36.86	11.12	214	20.58	5.41	176	17.09	4.56
Adductor	945	54.94	16.57	855	82.11	21.63	602	58.45	15.60
Total	***5701***	***331.45***	***99.99***	***3953***	***380.10***	***100***	***3858***	***374.77***	***100***

[a]Measurements were made on 1-mm slices illustrated in Fig. 7 using the software program, ITK-SNAP.[137]