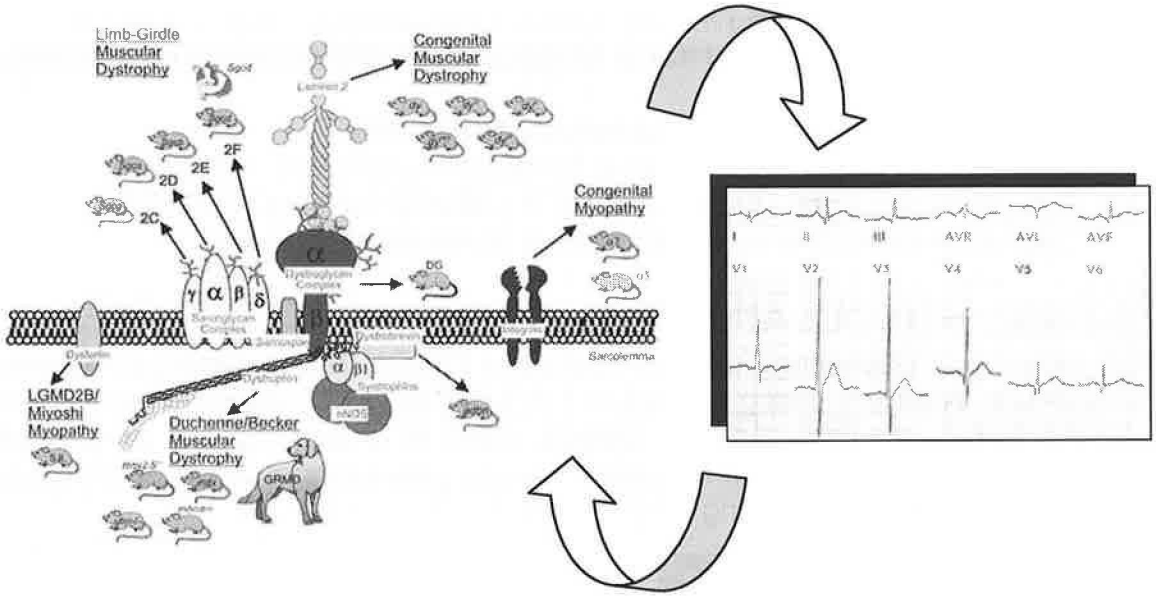
CARDIOMYOPATHY OF DYSTROPHINOPATHY:
From Bench to Bedside and Back and Forth



INTERNAL MEDICINE GRAND ROUNDS
UT Southwestern Medical Center

November 17, 2005

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Chief, Hypertension Division

Interests: Hypertension in Special Populations, Neural Control of Vascular Function

This is to acknowledge that Ronald Victor, MD has no financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Victor will be discussing off-label uses in his presentation.

CASE PRESENTATIONS

Case #1. Acute coronary syndrome in a 10 year-old boy.
Case #2. Dilated cardiomyopathy in a 65 year-old woman with myocardial infarction and normal coronary arteries.
Case #3. Acute viral myocarditis in a 37 year-old woman.

QUESTION: What disease-causing mechanism do these patients have in common?
ANSWER: Congenital (Case #1 and #2) or acquired (Case #3) disruption of dystrophin.

The learning objectives for these grand rounds are to describe:
1. How dystrophin deficiency leads to functional muscle ischemia.
2. The principal cardiac complications of the inherited dystrophinopathies.
3. Dystrophin's role in common forms of acquired heart disease.

DUCHENNE MUSCULAR DYSTROPHY

The first patient has Duchenne muscular dystrophy (DMD). This is the most common form of muscular dystrophy, being present in 1 in 3,500 boys. As originally described in 1851 by the British physician Edward Meryon, DMD is a crippling and progressive disease of boys leading to death in late adolescence. Two-thirds of cases are inherited as an x-linked trait and the remaining third are spontaneous mutations.

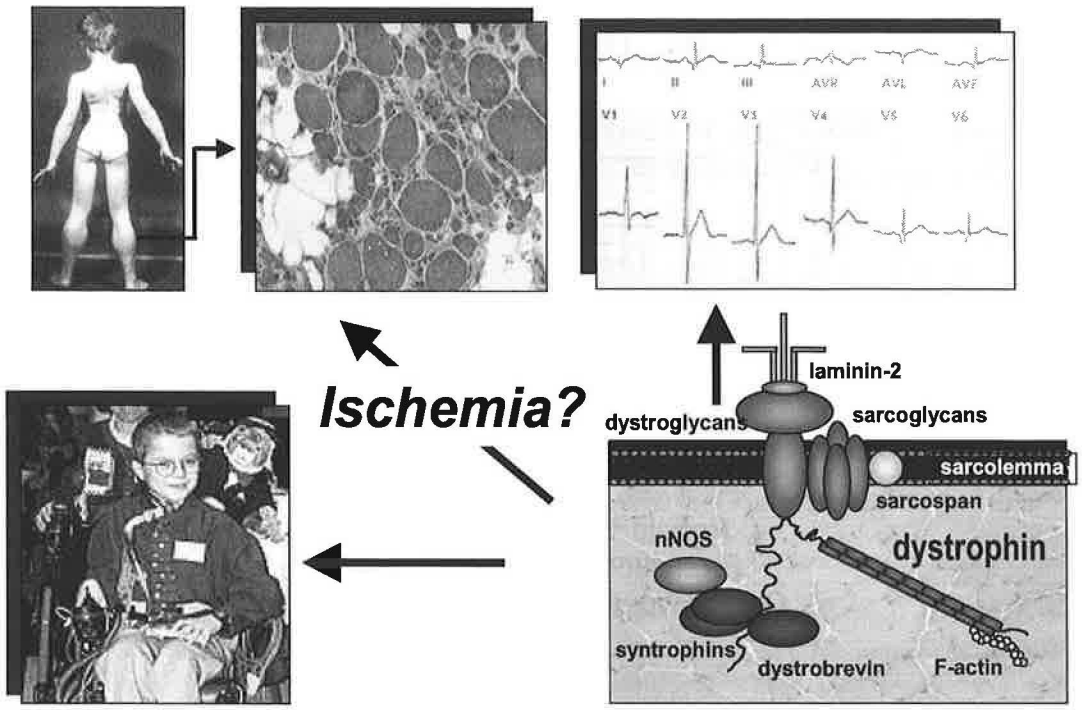

FIGURE 1. Dystrophin is the disease-causing gene in DMD. For review, see AEH Emery, *Lancet*, 2002.[1]

The boys are diagnosed as toddlers because of leg muscle weakness with calf pseudohypertrophy (muscle fibrosis). The classic EKG shows tall R waves in Leads V1-

V3 indicative of posterior-lateral wall fibrosis and the children usually die by age 20 of respiratory or cardiac muscle failure. With home mechanical ventilation, boys are living beyond age 20 and the proportion of deaths caused cardiac failure is increasing.

In 1987, dystrophin was discovered to be the gene mutated in DMD.[2] Excellent genetic mouse models have paved the way for elegant basic research on the dystrophin-glycoprotein complex.[3, 4] But we still have no treatment for this disease because we don't understand how dystrophin deficiency produces the clinical phenotype.

FUNCTIONAL MUSCLE ISCHEMIA IN DUCHENNE MUSCULAR DYSTROPHY

Dystrophin is a rod-like protein that provides a mechanical connection between the actin-based cytoskeleton and the extracellular matrix. As such, dystrophin is thought to function as a shock-absorber, maintaining sarcolemmal integrity during repeated muscle contraction. Loss of dystrophin destabilizes the sarcolemma. In addition, dystrophin deficiency also may disrupt transmembrane signaling. The dystrophin complex includes several proteins whose functions are incompletely understood. One of these is the neuronal isoform of nitric oxide synthase (nNOS). As nitric oxide (NO) is a potent vasodilator, NO deficiency could cause muscle ischemia. If this theory is correct, standard NO donors (nitroglyceride, nipride) should be therapeutic. Before discussing the cardiac disease, I will start with our own work demonstrating skeletal muscle ischemia in DMD. Dr. Gail Thomas and I are scientific partners on this work.

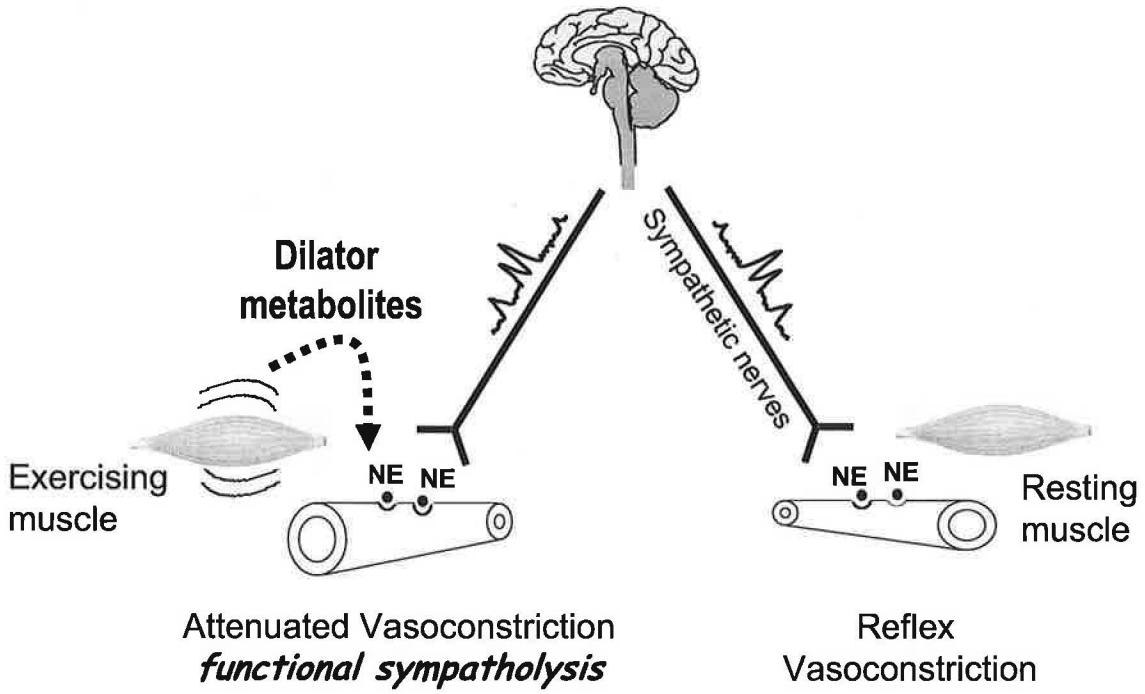

FIGURE 2. Functional sympatholysis during exercise. The classic paper describing functional sympatholysis was by Remensnyder JP, Mitchell JH, and Sarnoff SJ, *Circ Res*, 1962.[5]

The autonomic adjustments during exercise include reflex sympathetic activation in both resting and exercising skeletal muscle. In resting muscle, the release of norepinephrine (NE) from the sympathetic nerve endings causes α-adrenergic vasoconstriction, which shunts the cardiac output to the working muscle. In exercising muscle, however, adrenergic vasoconstriction is modulated by the local production of vasodilator metabolites. This functional sympatholysis is thought to optimize muscle perfusion. An impairment in this protective mechanism would render the exercising muscle ischemic.

So, we developed an animal model to identify the specific dilator metabolites involved.

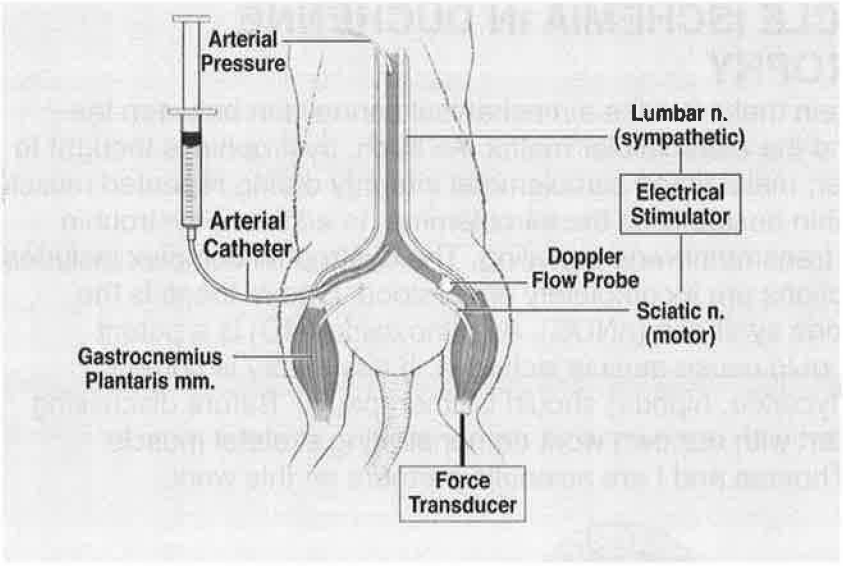

Figure 3. Anesthestized Rat Model.

In anesthetized rats, we measured blood flow responses to electrical stimulation of the sympathetic nerves in resting and contracting calf muscle. We also studied vasoconstrictor responses to adrenergic agonists infused directly into the arteries supplying the calf muscles.

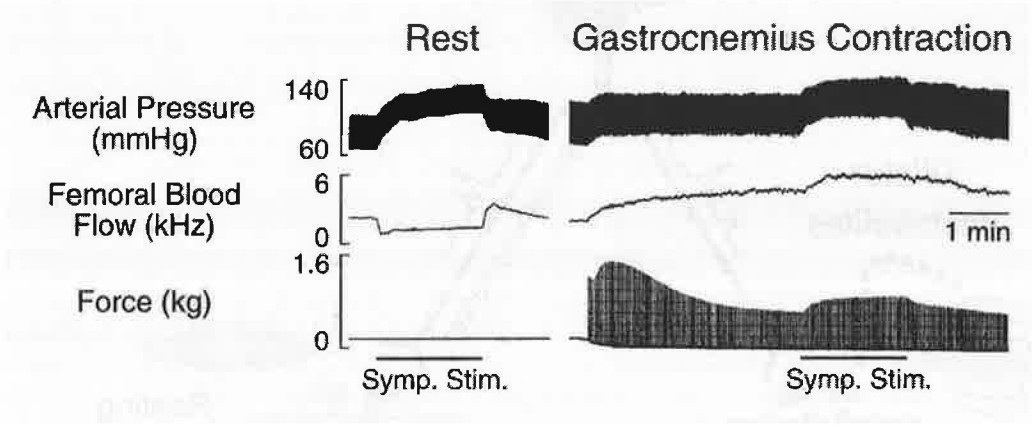

FIGURE 4. Experimental Protocol. From GD Thomas et al., *Am J Physiol*, 1994.[6]

In resting calf muscle, sympathetic stimulation increases blood pressure (BP) and decreases blood flow. This is sympathetic vasoconstriction. But when we superimposed the same sympathetic stimulus on fatiguing muscle contraction, blood flow does not fall but rather increases passively with the increase in BP. This is *functional sympatholysis*. Note that there is a partial restoration of force output, indirect evidence of improved muscle perfusion.

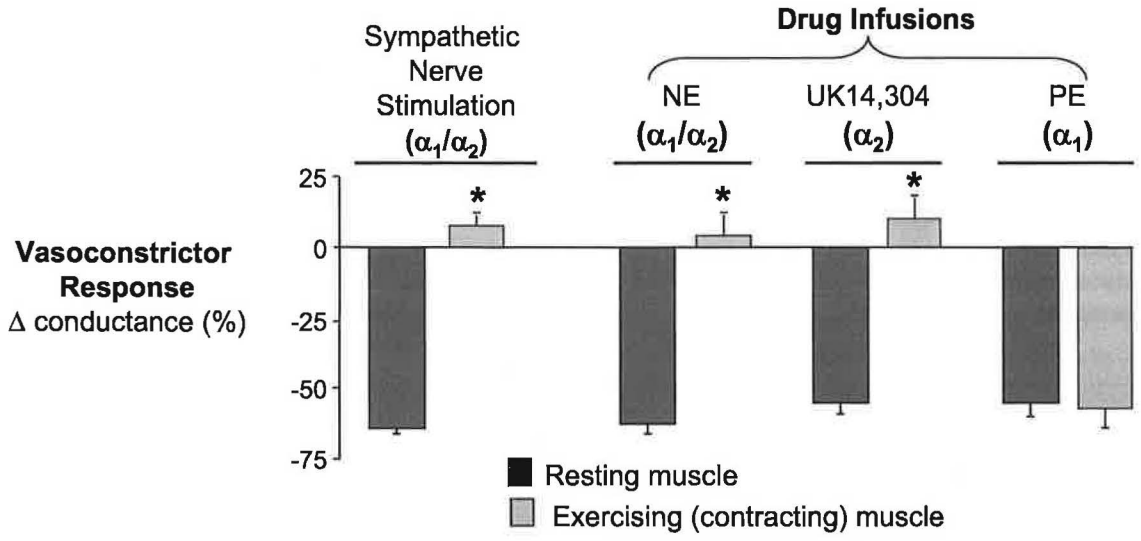

FIGURE 5. Summary data. From GD Thomas et al., *Am J Physiol*, 1994.[6]

Sympathetic vasoconstriction is greatly attenuated when superimposed on contraction-induced hyperemia. We then infused different adrenergic agonists into the arterial supply of the leg. Sympatholysis occurs post-junctionally at the level of the adrenergic receptor because norepinephrine (NE) mimicked the sympatholytic effect seen with sympathetic stimulation. The action is at the α2 adrenergic receptors because contraction eliminated the vasoconstrictor response to a specific α2 adrenergic agonist. The constrictor response to the α1 agonist phenylephrine (PE) was well preserved.

Our data are consistent with microcirculation studies in rat muscle.

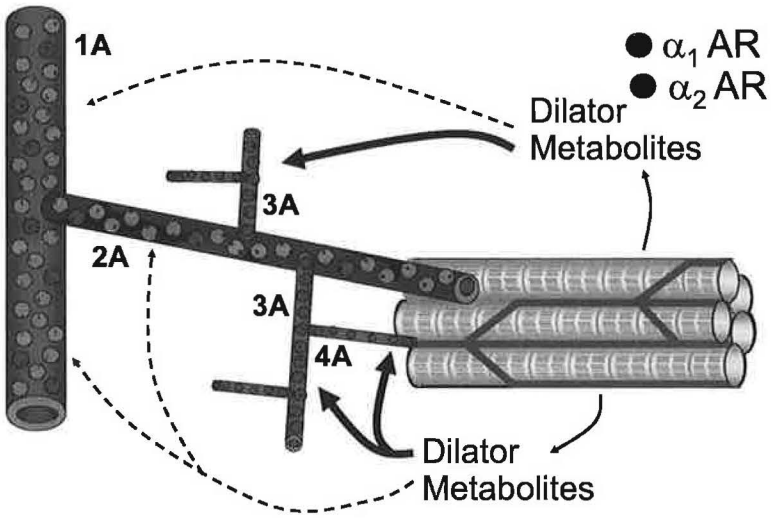

FIGURE 6. Distribution of α-adrenergic receptor (AR) subtypes in the vascular tree of skeletal muscle. From McGillivray-Anderson and Faber, *Circ Res*, 1990.[7]

The α2 ARs are preferentially distributed on the most distal parts of the microcirculation where they are directly accessible to production of vasodilator metabolites. In contrast, α 1 ARs are mainly located in the more proximal resistance arterioles where they are not so accessible to these metabolites. During exercise, this scenario could optimize nutrient flow while maintaining the tone of the resistance vessels and therefore BP.

NO was a particularly attractive candidate because the neuronal isoform of NOS is highly enriched in skeletal muscle where it is part of the dystophin glycoprotein complex, which targets the enzyme to the sarcolemma.

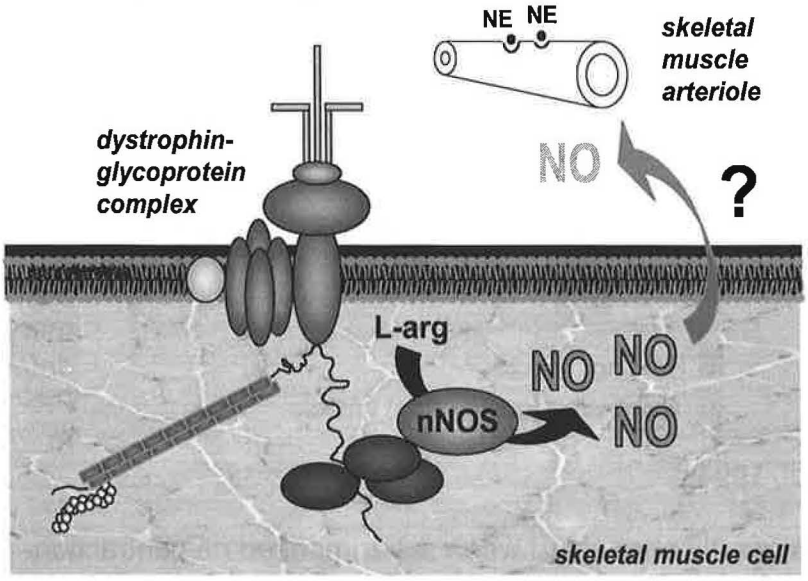

FIGURE 7. Skeletal muscle-derived NO as the mediator of sympatholysis.

This sarcolemmal location accomplishes at least two important functions: 1) it coordinates Ca^{2+}-dependent activation of the enzyme to the contraction-induced Ca^{2+} signal, and 2) it places the NO formed right at the cell surface where is has a short distance to diffuse to the adjacent vascular smooth muscle.

We performed several different experiments to test this hypothesis and began by asking whether sympatholysis is sensitive to inhibition by L-NAME, a potent NOS inhibitor that has promiscuous effects on all NOS isoforms—nNOS as well as eNOS and iNOS.

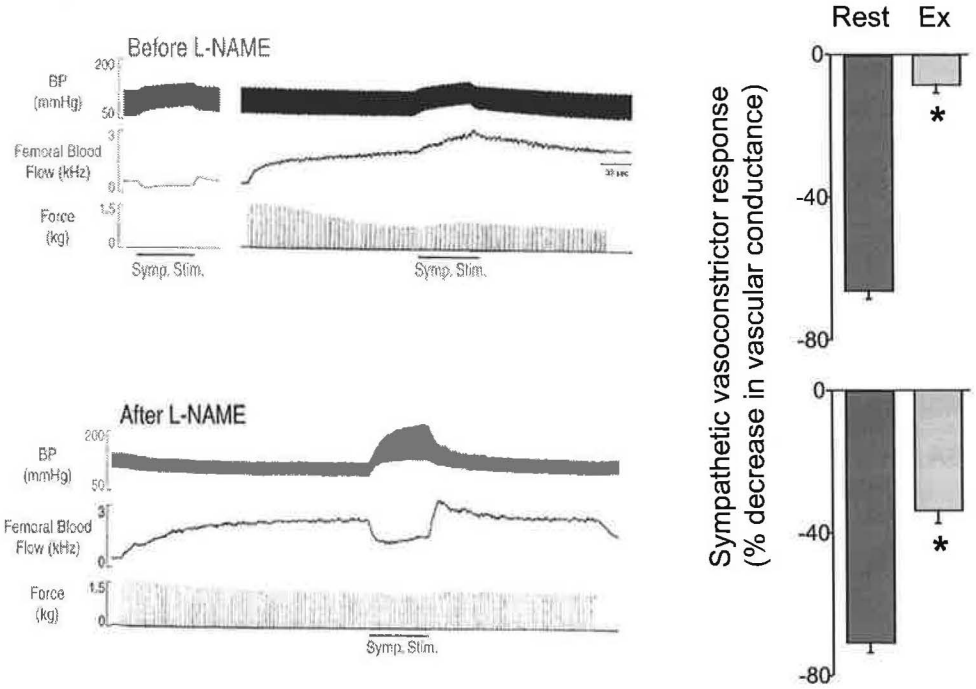

FIGURE 8. Role of nitric oxide synthase. Left panel, experimental data before and after intravenous L-NAME in one rat. Right panel, summary data. From GD Thomas and RG Victor, *J Physiol Lond*, 1998.[8]

In rats, L-NAME partially reversed sympatholysis both with electrical stimulation of the sympathetic nerves and infusion of an α AR agonist. Before L-NAME, blood flow increases when sympathetic stimulation is superimposed on hindlimb muscle contraction. After L-NAME, the same sympathetic stimulus in the same rat causes a sharp decrease in blood flow to the contracting muscle.

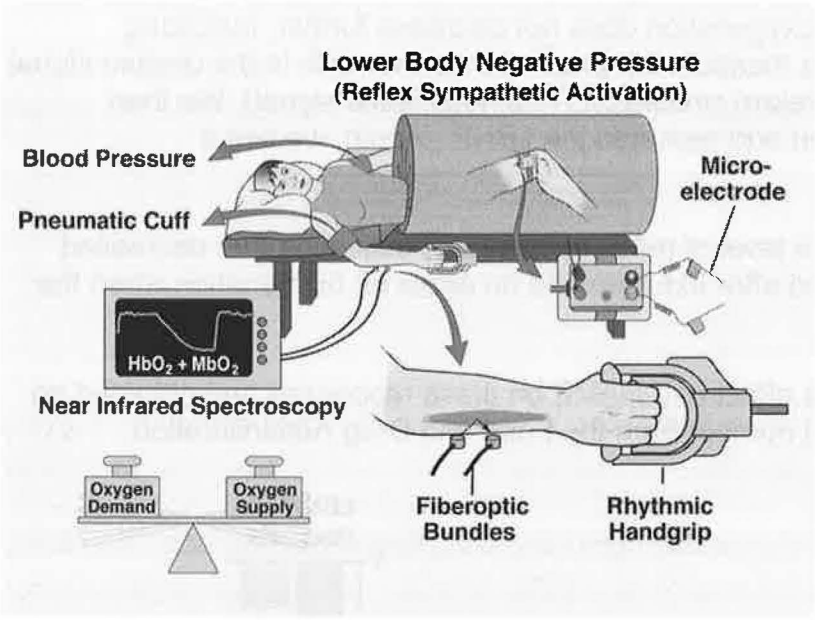

FIGURE 9. Experimental set-up for translational studies in human subjects.

This is a busy slide but simply put we used simulated orthostatic stress with lower body negative pressure (LBNP) to cause reflex sympathetic activation in resting and exercising forearm muscle and used near infrared (NIR) spectroscopy to measure muscle oxygenation at the level of the microcirculation. The principal is that laser light in the 600 to 900 nM range is absored by the heme-porphyrin complex of the hemoglobin and myoglobin molecules causing a shift in peak absorption for the oxygenated and de-oxygenated species. So, the redox state provides an index of tissue oxygen supply relative to demand. We used microelectrode recordings to quantify the sympathetic stimulus.

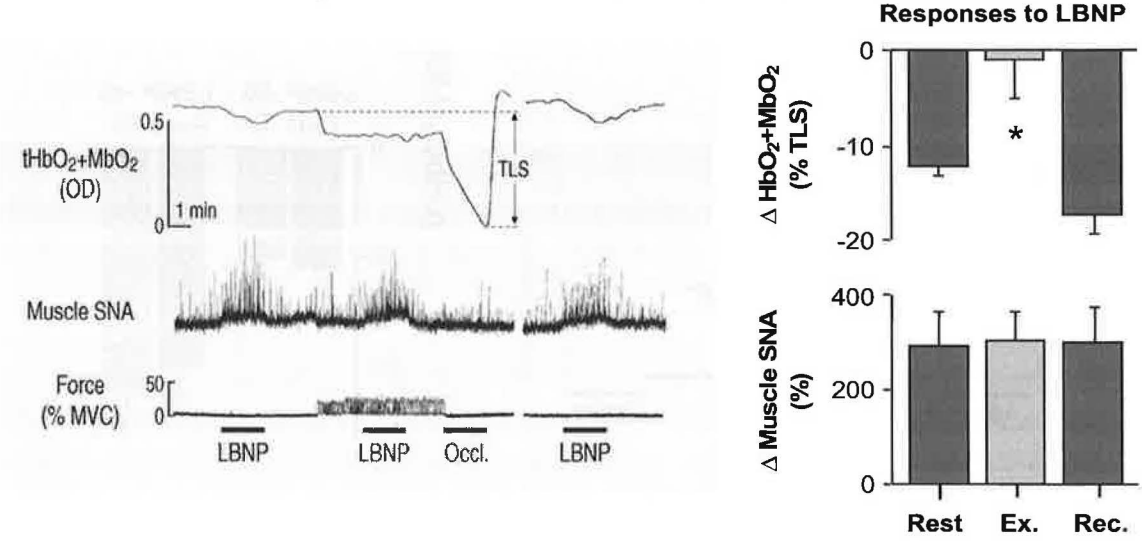

FIGURE 10. Functional sympatholysis in exercising human skeletal muscle. Left panel, illustrative experiment in one healthy subject. Right panel, summary data. From J Hansen et al., *JCI*, 1996.[9]

On the original record, the panels are the tissue oxygenation signal, sympathetic nerve activity (SNA), and handgrip force. At rest, sympathetic activation produces a decrease in muscle oxygenation, indicating adrenergic vasoconstriction. During handgrip exercise at 20% maximal voluntary contraction (MVC), the oxygen signal decreases as the muscle is consuming oxygen. When we superimposed the reflex sympathetic stimulus on this mild exercise, muscle oxygenation does not decrease further, indicating sympatholysis. Responses are measured in proportion to the nadir in the oxygen signal during cuff occlusion of the forearm circulation (TLS, total labile signal). We then restored the forearm circulation and repeated the LBNP. Again, we see a vasoconstrictor response.

The summary data show that a level of reflex sympathetic activation that decreased muscle oxygenation before and after exercise had no effect on oxygenation when the muscle was exercised.

Now we were ready to test the effect of L-NAME on these responses and obtained an investigational new drug (IND) number from the Food and Drug Administration.

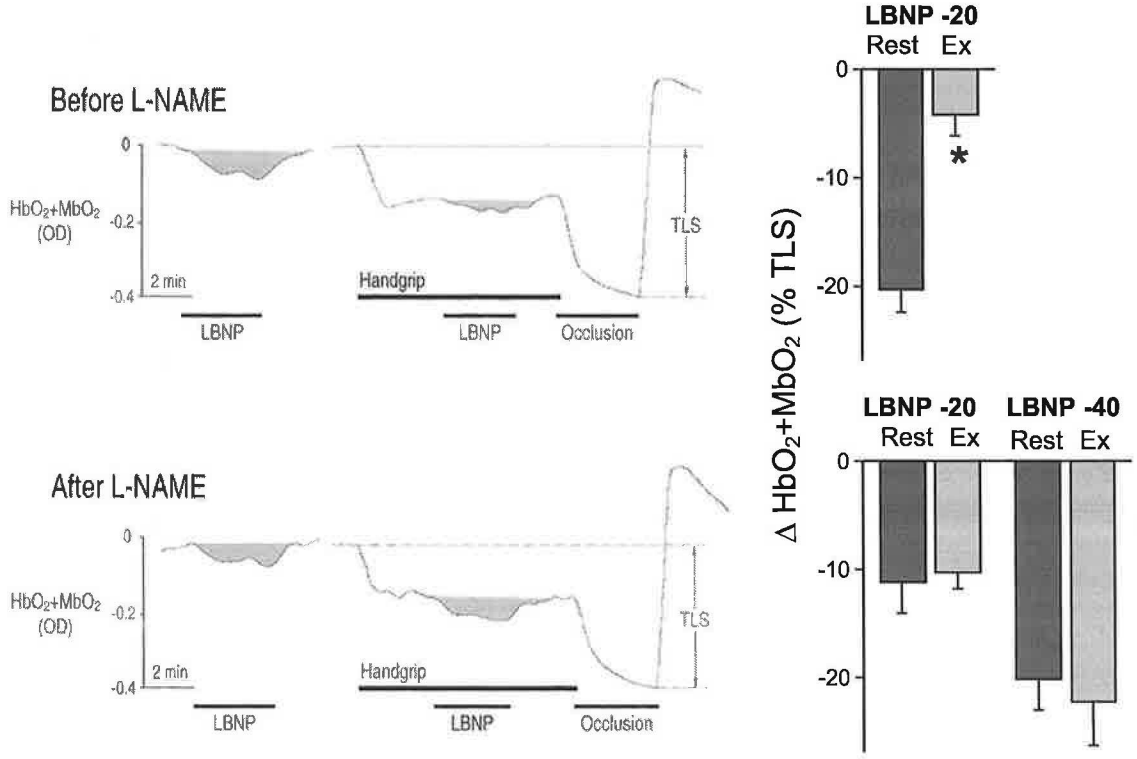

FIGURE 11. Evidence for NOS modulation in humans. Left panel, illustrative experiment in one healthy subject. Right panel, summary data. From B Chavoshan et al., *J Physiol Lond*, 2002.[10]

L-NAME blunted sympatholysis in humans as well as rats.

We then miniaturized our techniques and developed a parallel model in mice.

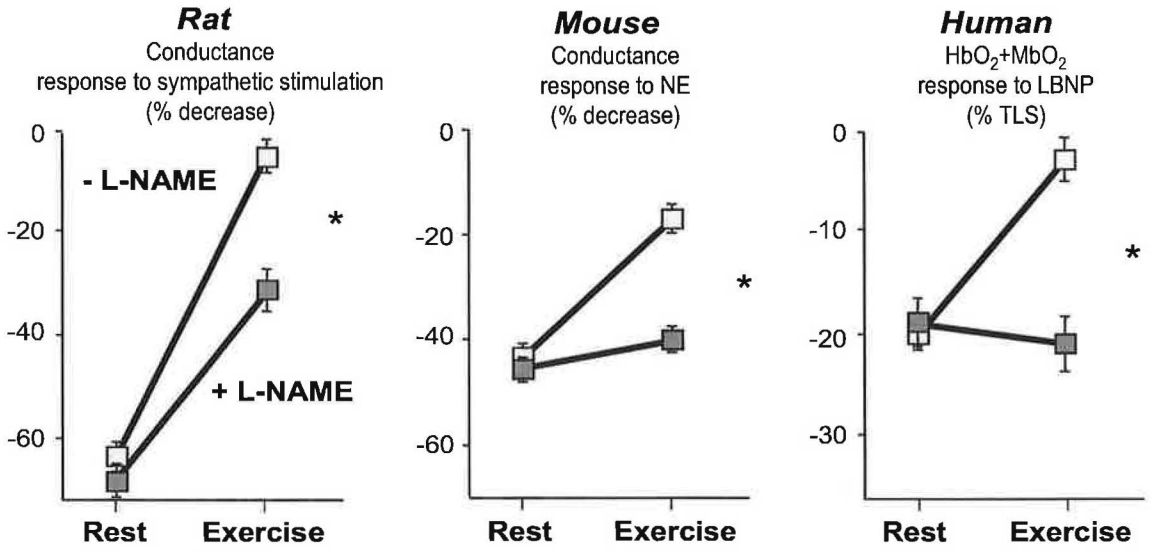

FIGURE 12. Parallel results in three species.

We now were ready to study mutant mice and determine if the robust L-NAME effect is due to blockade of nNOS rather than eNOS.

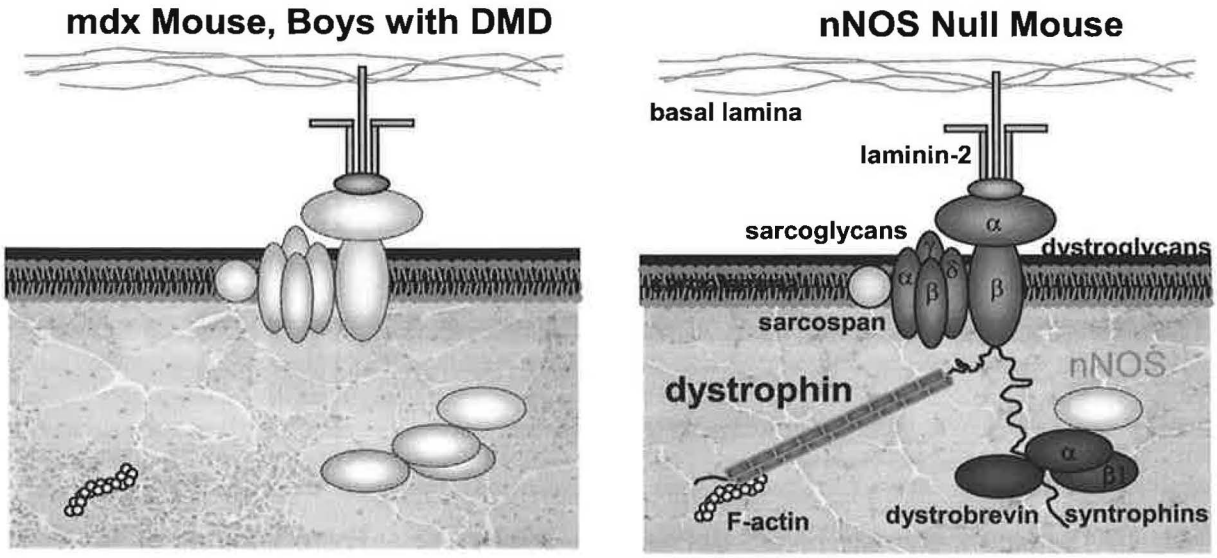

FIGURE 13. Genetic models. Left panel, Dystophin-deficient skeletal muscle is characterized by complete loss of all the dystrophin-associated proteins including nNOS. Right panel, all other dystrophin proteins are normal in the nNOS null mouse.

Jim Stull's lab at UT Southwestern was one of the first to show that nNOS is reduced in the muscles of mdx and of boys with DMD.[11] In an in-situ muscle preparation they showed that arteriolar dilation during exercise was attenuated in mdx mice.

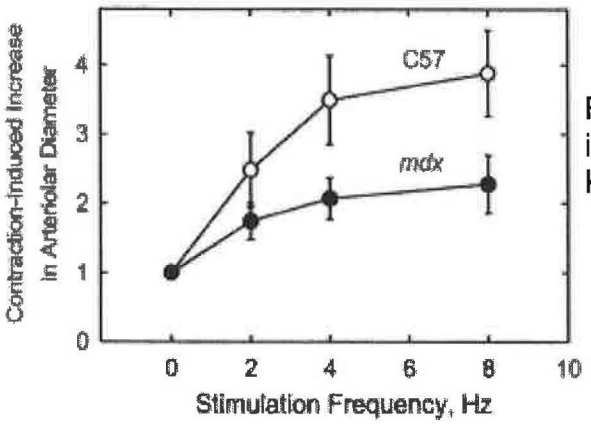

FIGURE 14. Exercise-induced arteriolar dilation is attenuated in nNOS-deficient mdx mice. From KS Lau et al., *FEBS Lett.*, 1998.[12]

Remarkably, sympatholysis was absent both in the mdx mouse as well as in the nNOS null mouse, implicating a pivotal role for skeletal muscle-derived NO.

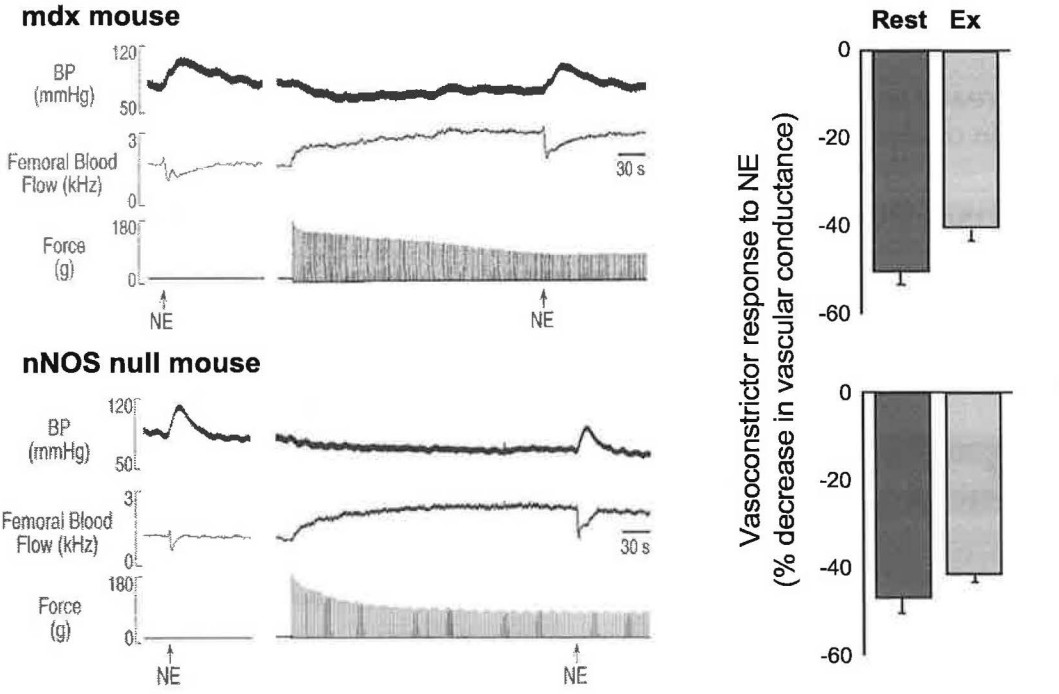

FIGURE 15. Phenoytype in nNOS-deficient skeletal muscle. From Thomas et al., *PNAS*, 1998.[13]

We then performed translational experiments in children with muscular dystrophy. We collaborated with Dr. Susan Iannaccone, who has developed a very large and well characterized population of children with muscular dystrophies in Dallas. Here are data from a dozen 8-10 year-old boys with DMD and age-matched healthy controls.

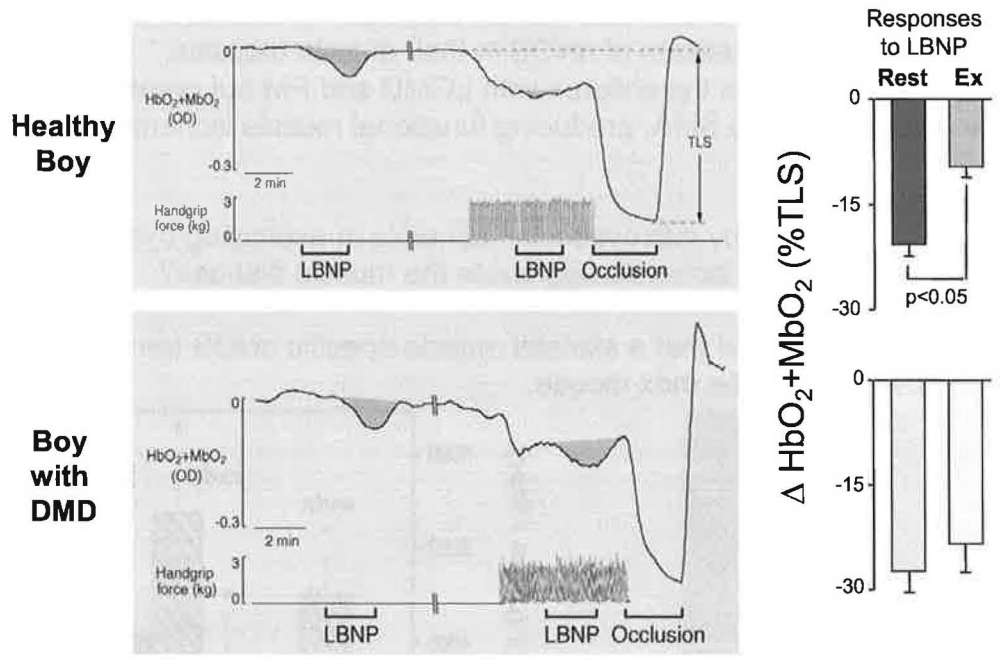

FIGURE 16. Functional ischemia in boys with DMD. M Sander et al., *PNAS*, 2000.[14]

As in the mdx mice, sympatholysis was impaired in the boys with DMD. In these experiments, handgrip plus LBNP at -20mmHg simulates the condition of mild arm exercise in a child seated in a wheel chair. We then studied disease controls.

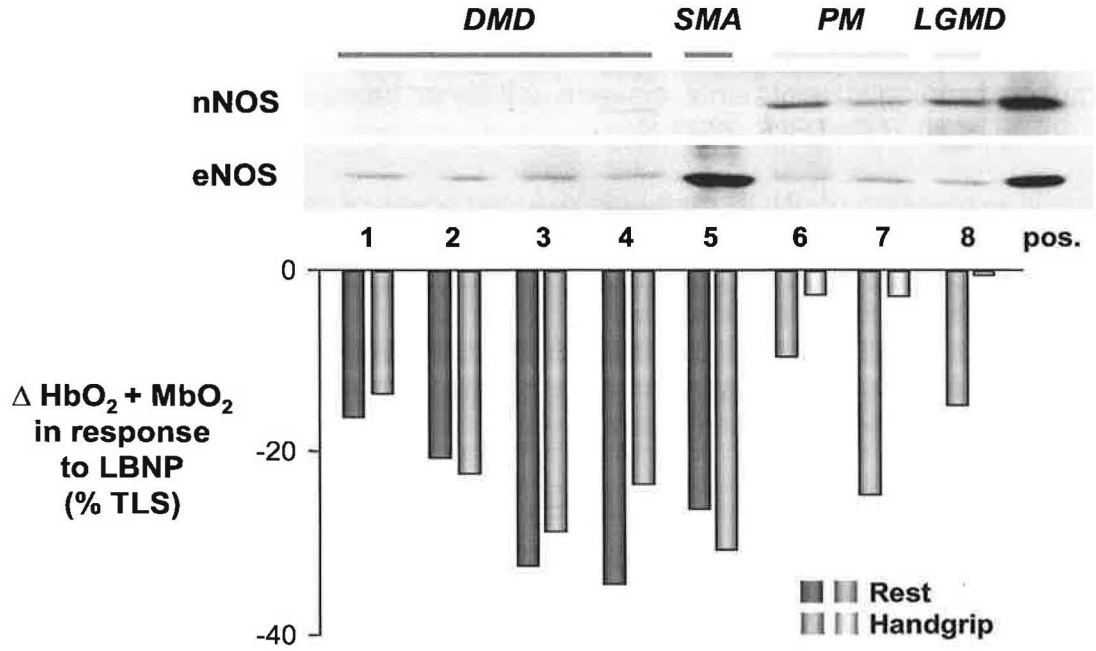

FIGURE 17. Boys with DMD vs. disease controls. Top panel, Western blot experiments in the children's muscle biopsies. Bottom panel, corresponding functional responses. SMA, spinal muscular atrophy; PM, polymyositis, LGMD, limb-girdle muscular dystrophy From Sander et al., *PNAS*, 2000.[14]

Expression of eNOS is comparable in all these muscle biopsies. The functional responses segregated with the expression of nNOS in their muscle biopsies. Sympatholysis was clearly evident in the children with LGMD and PM but greatly attenuated not only in DMD but also SMA, producing functional muscle ischemia (light blue bars).

So, NO deficiency is accompanied by microvascular ischemia in exercising dystrophin-deficient skeletal muscle. Does this ischemia aggravate the muscle disease?

James Tidball's lab at UCLA showed that a skeletal muscle-specific nNOS transgene partially rescues the phenotype in the mdx mouse.

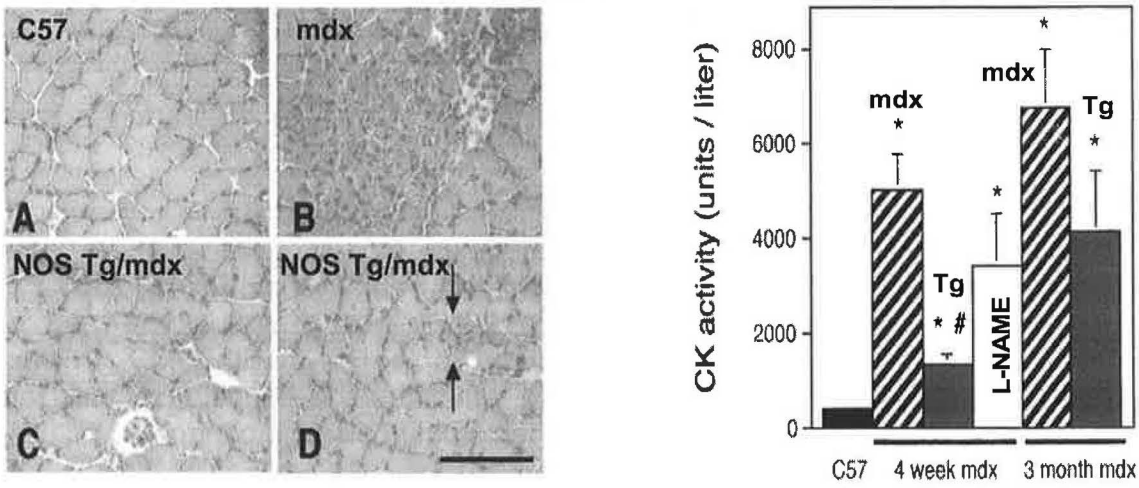

FIGURE 18. Transgenic (Tg) rescue of dystrophic skeletal muscle in mdx mice. Left panel, muscle histology. Right panel, disease activity as measured by blood CK levels. From Wehling et al., *J Cell Biol*, 2001.[15]

Transgencic expression of nNOS markedly reduced areas of muscle inflammation and fibrosis and partial normalization of CK blood levels. The therapeutic benefit waned with age and we hypothesize that the protection afforded could be even greater if the nNOS were targeted directly to the sarcolemma.

So far, we have focused all our attention on exercising skeletal muscle. But dystrophin also is abundant in vascular smooth muscle.

LARGE ARTERY &VEIN **SMALL ARTERY & VEIN**

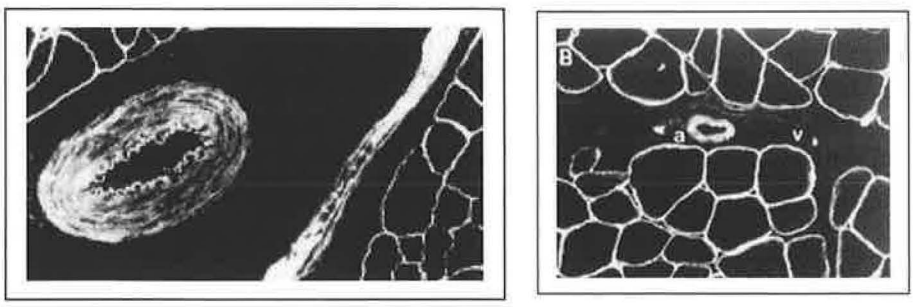

FIGURE 19. Immunohistochemistry experiments with a monoclonal antibody against dystrophin. From Rivier et al, *FEBS Lett*, 1997.[16]

As shown above, dystrophin is normally expressed in vascular smooth muscle of large arteries and veins and in small arteries but not in small veins. We therefore went back to our translational studies and found augmented sympathetic arterial vasoconstriction in resting human skeletal muscle.

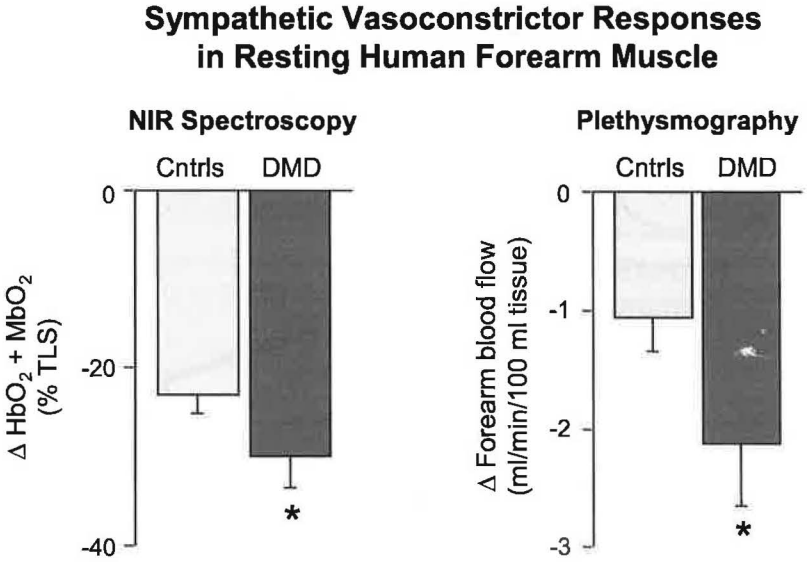

FIGURE 20. Augmented reflex vasoconstriction in DMD. From Sander et al., PNAS, 2000.[14]

In resting forearm muscle, LBNP at -20 elicted a greater vasoconstrictor response in boys with DMD than in healthy age-matched controls as measured either by NIR spectroscopy or by plethysmography.

So, we have started to look for this in our mdx mice.

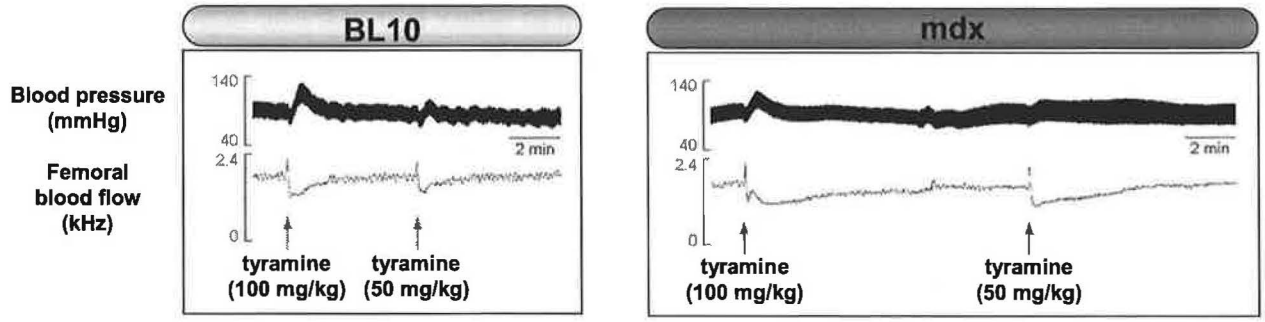

FIGURE 21. Vasoconstrictor responses to tyramine (indirect sympathomimetic) in a wild type (BL 10) mouse and a mdx mouse. Unpublished data from GD Thomas.

This experiment shows the decrease in hindlimb muscle blood flow evoked by intraarterial infusion of tyramine in a control mouse and a mdx mouse. Although the peak decrease in flow is the same, the duration of the vasoconstrictor response is more than tripled in the mdx mouse.

So, our working hypothesis is as follows.

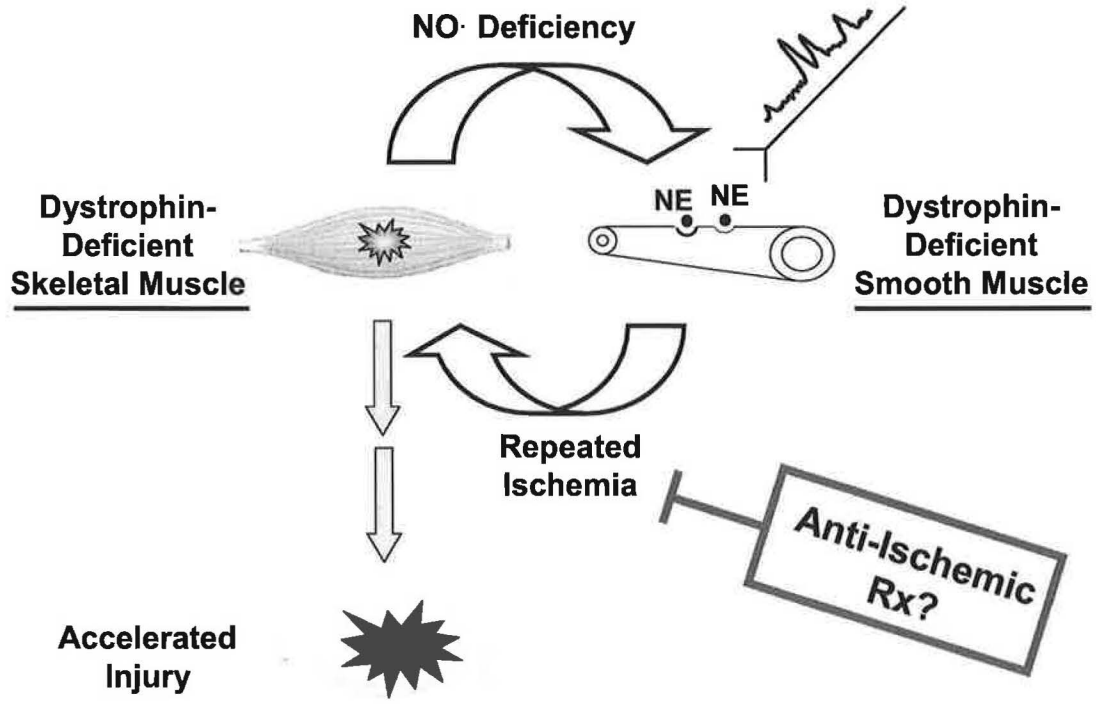

FIGURE 22. Double hit (Texas Two-Step) hypothesis.

First, dystrophin deficiency produces a primary structural defect. Second, the abnormal muscle fibers are subjected to repeated bouts of ischemia because adrenergic vasoconstriction is unopposed and elicits an exaggerated vasoconstrictor response due to an instrinsic defect in dystophin-deficient vascular smooth muscle This cycle occurs during physiologic and pathophysiologic stimuli to reflex sympathetic activation: exercise, upright posture, increased work of breathing, respiratory infections, and non-cardiac surgery. So, we think that repeated ischemic insult is not the primary defect but adds insult to injury. If so, this theory provides a new conceptual framework for recommending anti-ischemic therapy to slow disease progression. This would not be a cure and would need to be started in children identified from birth. Based on our studies, existing sympathloytics, NO donors, and K_{ATP} channel openers should be effective but muscle specific delivery may be necessary to avoid systemic hypotension.

Now lets turn to the heart ...

CARDIOMYOPATHY OF DUCHENNE MUSCULAR DYSTROPHY AND RELATED DYSTROPHINOPATHIES

This topic has been recently reviewed.[17] In DMD, limb muscle weakness dominates the clinical picture through the first 10 years of life and children are usually wheel chair-bound by age 12. In the early teens, the dominant symptoms are related to progressive respiratory muscle weakness. Nocturnal mechanical ventilation has been associated with an increase in mean life expectancy from 14 years in the 1960s to 25 years after 1990.[18] As affected boys are living longer, almost all develop some degree of

cardiomyopathy, which has emerged as the second most common cause of death in DMD (30% of cases). The pathologic hallmark of the cardiac involvement is fibrosis beginning preferentially in the posterolateral wall of the left ventricle, producing the characteristic tall R-waves in the anterior precordial leads and deep narrow Q-waves in the anterolateral leads. This site-specificity is an enigma.

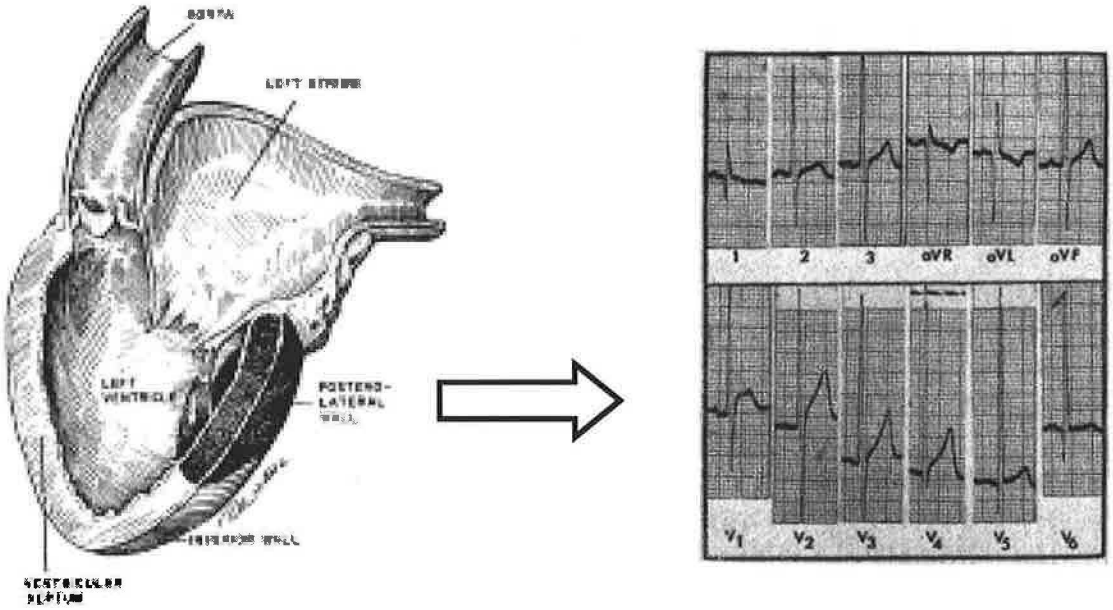

FIGURE 23. Characteristic pathologic and ECG findings in DMD. Left panel, diagram of the left side of the heart illustrating the typical distribution of the fibrous scarring at necropsy. Right panel, corresponding ECG showing tall precordial R-waves and deep narrow Q-waves in the lateral leads. From JK Perloff et al., *Am J Med*, 1967.[19]

Early cardiac abnormalities that predate the onset of failure include frequent PVCs, atrial arrhythmias, and unexplained sinus tachycardia. Progressive patchy cardiac fibrosis leads to life-threatening ventricular tachyarrhythmia and eventually full blown dilated cardiomyopathy. Because the adolescents are wheel-chair bound and thus physically inactive, cardiac symptoms often are minimal until systolic failure is far advanced. Standard treatment of heart failure with ACE inhibitors, diuretics, and β-blockers may improve cardiac function in DMD but has not been shown to prolong life.[20] Cardiac transplantation is generally contraindicated because of advanced chest wall deformities (kyphoscoliosis) and respiratory failure.

Cardiac failure predominates the clinical picture and is the main cause of death in *Becker's muscular dystrophy*, an X-linked trait in which dystrophin production is partially reduced but not absent. The limb and respiratory muscles are stronger than in Duchenne's and so the boys remain active into the third and fourth decades of life. Congestive heart failure is often the presenting diagnosis. The associated skeletal muscle disease often is discovered subsequently during the medical evaluation (mild muscular weakness, elevated serum CK levels). There are case reports of adults with Becker's muscular dystrophy who have undergone successful cardiac transplantation.

X-Linked Dilated Cardiomyopathy is a very rare dystrophinopathy in which dystrophin is selectively absent in the myocardium. Patients typically present with rapidly progressive heart failure in adolescence or early 20s. Without a careful family history, the diagnosis is easily missed because heart failure dominates the clinical picture, with little or no skeletal muscle involvement.

There is unequivocal evidence that ~10% of female carriers of dystrophin mutations develop overt cardiac failure even in the absence of any skeletal muscle involvement.[21] Known carriers should undergo regular non-invasive cardiac testing to make an early diagnosis of sub-clinical cardiomyopathy. In one study of 56 asymptomatic carriers (mean age 45) and 35 asymptomatic non-carriers (mean age 35) from the same families, previously unrecognized abnormalities on ECG or echocardiogram were found in 18% of the carriers but in none of the controls. Echocardiograms showed dilated cardiomyopathy in 7% of the carriers.

Thus, inherited dystrophinopathy in female carriers constitutes an important but often unrecognized form of heart disease in women. The inherited defect provides a weakened cardiac reserve to withstand common acquired adult heart diseases.

There are an increasing number of case reports of female carriers with class IV heart failure who have undergone successful cardiac transplantation. Important questions are whether a) newer imaging modalities will permit earlier detection of sub-clinical cardiac disease, b) genetic testing can identify rapid progressors, and c) whether effective prophylactic therapeutics can be developed to slow or prevent progression of the cardiac disease. This is a fertile area for multi-disciplinary research, including multi-center prospective treatment trials. We are working with Dr. Ron Peshock to determine the sensitivity of gadolinium-MRI to detect cardiac scarring in asymptomatic carriers. The mdx mouse is a reasonably good model for the cardiomyopathy of DMD except that the mdx cardiac phenotype is much milder. Tidball's lab recently extended their work to show that a nNOS transgene rescues the cardiac phenotype in the mdx mouse.

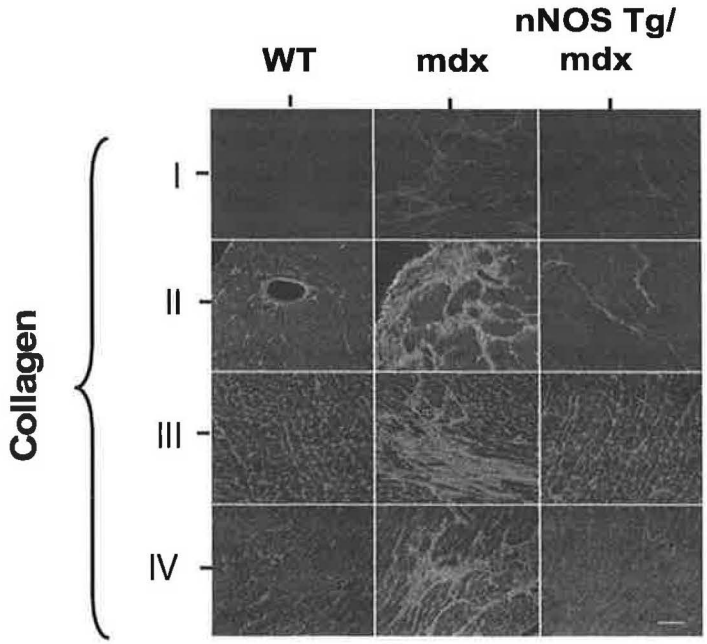

FIGURE 24. Normalization of cardiac histology in the mdx mouse by transgenic over-expression of nNOS. From M Wehling-Henricks et al., *Human Molecular Genetics*, 2005.[22]

Here they show that a nNOS transgene targeted to cardiac muscle prevents the extensive cardiac fibrosis which is the histologic hallmark of the cardiomyopathy. The transgenic experiments also rescued the ECG abnormalities. These include deep Q-waves and sometimes a right bundle branch block pattern.

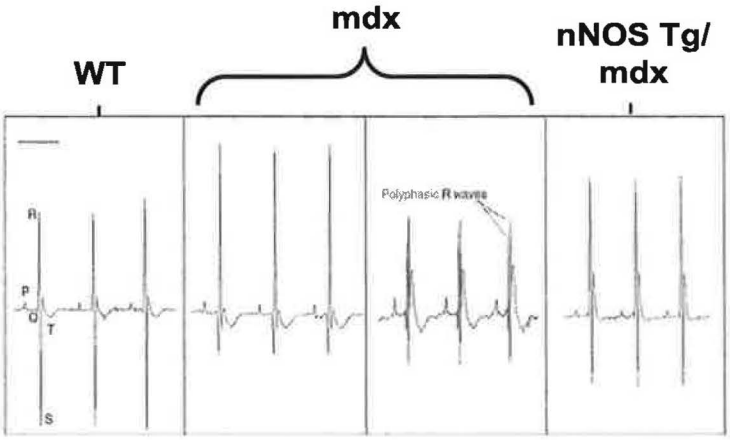

FIGURE 25. From M Wehling-Henricks et al., *Human Molecular Genetics*, 2005.[22]

The mechanism underlying these impressive findings remains to be determined. The authors believe that inflammation plays a pivotal role in disease progression and they provided evidence that NO is anti-inflammatory in this setting.

In addition, nNOS normally is expressed in cardiac vagal nerve terminals where NO acts locally to accelerate the release of acetylcholine, thereby augmenting vagal slowing of heart rate and maintaining normal heart rate variability.

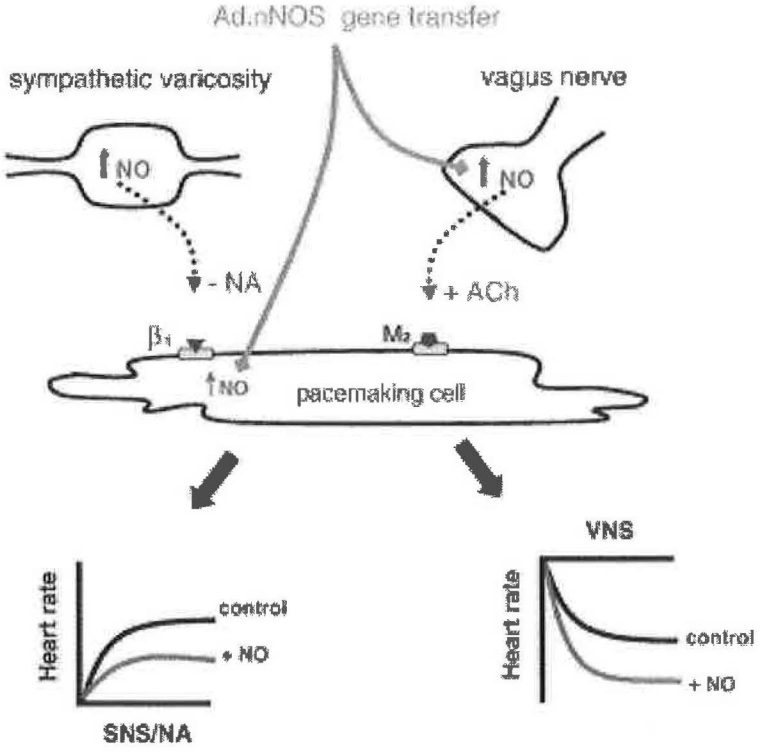

FIGURE 26. Gene transfer experiments provide evidence that nNOS expression in the cardiac vagus nerve and heart increases the vagal influence on the sinus node while decreasing the sympathetic influence. From RM Mohan et al., *Prog Biophys Mol Biol*, 2004.[23]

Thus, NO deficiency constitutes one potential explanation for the unexplained sinus tachycardia and decreased heart rate variability in boys with DMD, even young boys with preserved left ventricular function. Jason Chang, a Doris Duke student fellow from Kentucky University, is working with us to test this hypothesis. The persistent sinus tachycardia increases myocardial oxygen demands. In the mdx mouse, transgenic expression of nNOS normalized sinus rate and heart rate variability.

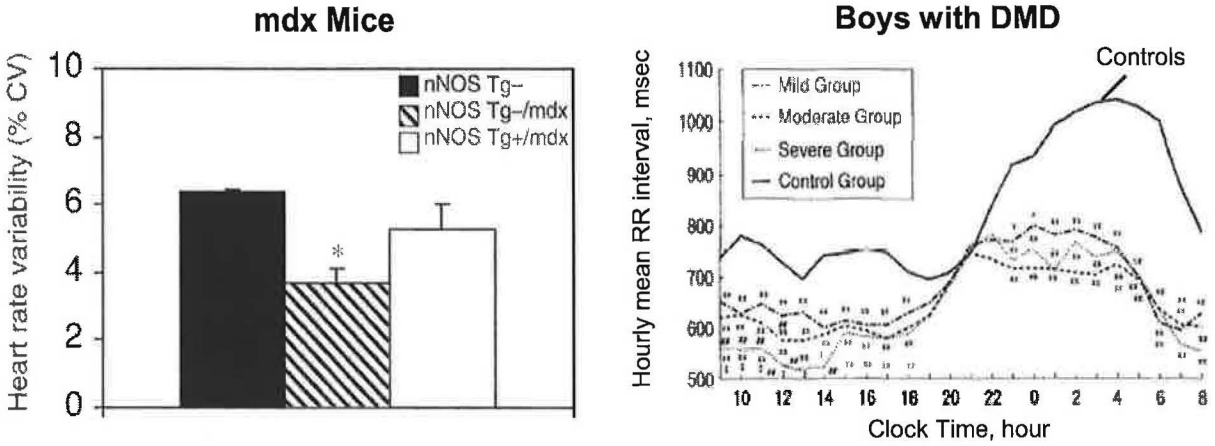

FIGURE 27. Potential role for nNOS in the regulation of cardiac vagal tone. Left panel, transgenic expression of nNOS rescues heart rate variability in mdx mice. From M Wehling-Henricks et al., *Human Molecular Genetics*, 2005.[22] Right panel, sinus tachycardia and impaired nocturnal decline in heart rate even in boys with mild DMD. From M Yotsukura et al., *Am J Cardiol*, 1995.[24]

In addition, we believe that cardiac NO deficiency leads to unopposed α-adrenergic vasoconstriction in the coronary microvessels. In other words, we hypothesize an ischemic component to what has been assumed to be a non-ischemic form of dilated cardiomyopathy. Drs. Claudio Ramaciotti, William Scott and colleagues at Children's Hospital of Dallas, recently reported a pair of unrelated 10-year old boys with DMD who presented with chest pain and an "acute coronary syndrome."[25] This is the first case presented at the beginning of the grand rounds.

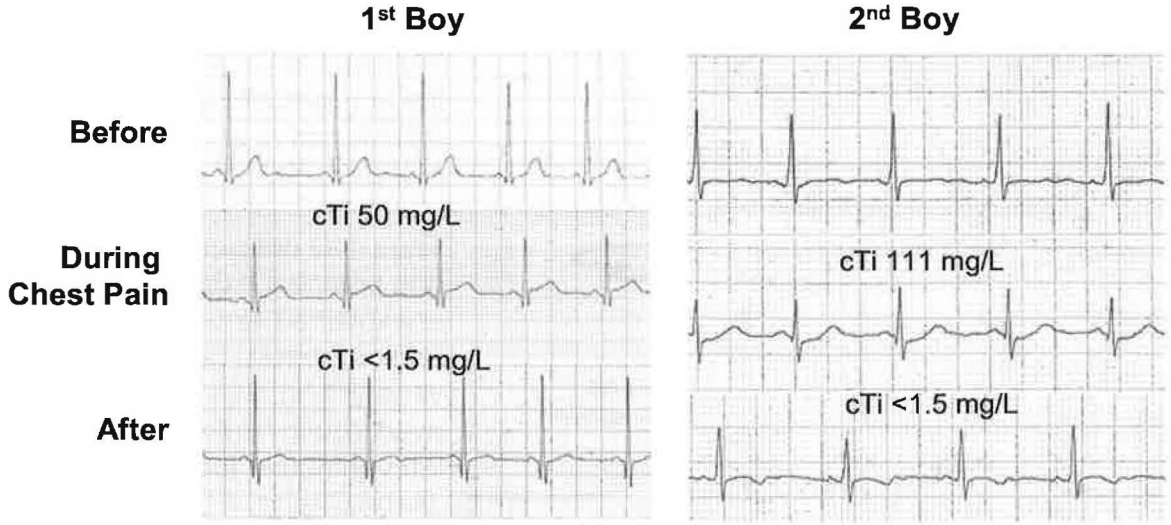

FIGURE 28. "Acute coronary syndrome" in unrelated boys with DMD. From C Ramaciotti et al., *Pediatric Cardiology*, 2003.[25]

"Acute coronary syndrome" is in quotation marks because at least in one child they enlisted the help of the director of our adult cardiac cath lab, Dr. Joaquin Cigarora, to

show that the coronary arteries were completely clean of CAD as you would expect. The first boy presented with rather mild ST-segment elevation but a large tropinin leak that resolved after the acute event. The acute troponin leak was even larger in the second child who presented with ST-segment depression and pseudo-normalization of the T- waves. It has been proven that cTnI and cTnT can be elevated in isolated boys with DMD and leg muscle biopsies show that the regenerating skeletal muscle is not the source. By process of elimination, this reflects acute injury to cardiac muscle. The true prevalence of CTnI elevations in DMD is an important unanswered question.

There are other isolated case reports of cardiac stunning producing acute severe hypotension, tachycardia, and heart failure during non-cardiac surgery in boys with DMD and young adults with Beckers' muscular dystrophy. Female carriers of x-linked dilated cardiomyopathy generally present with chest pain. The second patient I presented is the mother of a boy who died with DMD. She had an acute myocardial infarct at age 65 but was found to have normal coronaries. Our theory is that these patients are subject to repeated bouts of asymptomatic myocardial ischemia leading over time to piecemeal necrosis. If so, anti-ischemic and sympatholytic therapy should slow disease progression. On the other hand, β-blockade might further unmask α adrenergic vasoconstriction and augment the ischemic injury. More research is needed.

CARDIOMYOPATHY OF LIMB GIRDLE MUSCULAR DYSTROPHY

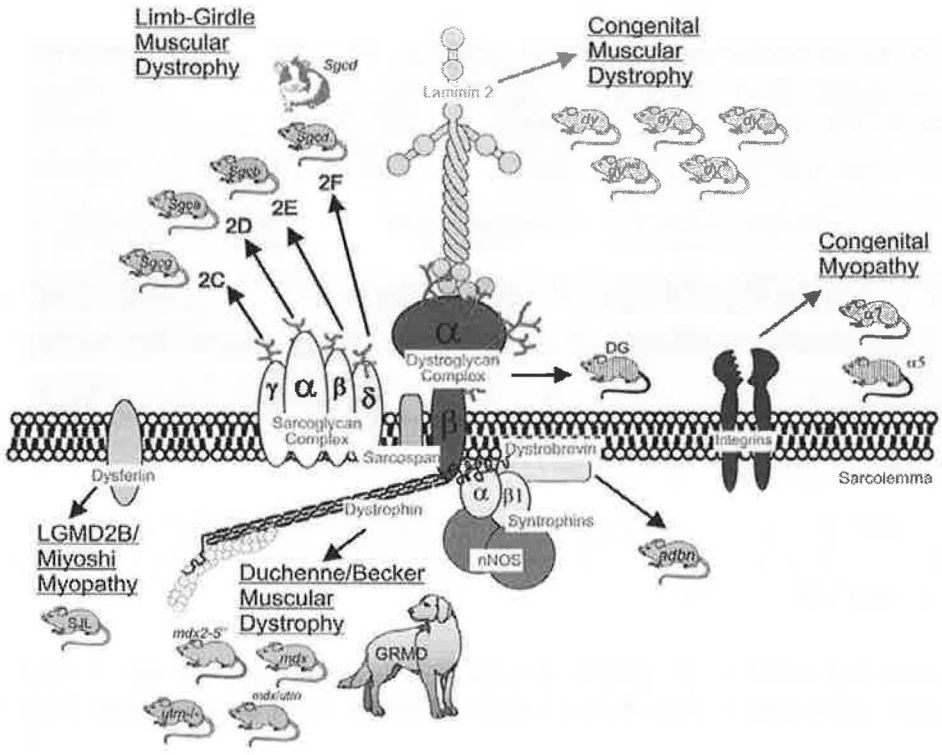

FIGURE 29. Animal models of the various dystrophinopathies. (From V Allamand and KP Campbell, *Human Molecular Genetics*, 2000.[26]

Limb-girdle muscular dystrophies (LGMDs) are a group of disorders that are characterized by progressive muscle weakness starting in the shoulders and pelvic girdle. They mainly have autosomal recessive inheritance and are due to mutations in the sarcoglycans, one of the major dystrophin-associated glycoproteins. Dystrophin itself is intact. Highly specific mouse models are shedding new light on the normal function of the sarcoglycan complex in both striated and smooth muscle.

Recently an increasing number of patients withy sarcoglycanopathies have been reported to exhibit cardiomyopathy.[27, 28] All the sarcoglycans shown in the figure below are expressed in skeletal and cardiac muscle. The β and δ sarcoglycans also are expressed in smooth muscle whereas α sarcolycan is not. Mice deficient in α sarcoglycan develop skeletal muscle dystrophy but their hearts are normal. In contrast, mice deficient in β or δ sarcoglycan develop cardiomyopathy as well as muscular dystrophy.

Kevin Campbell's lab at the University of Iowa has provided compelling evidence that this is an ischemic form of cardiomyopathy. Young mice develop spontaneous coronary vasospasm with elevated cTnI levels prior to the development of cardiomyopathy. As the mice age, the hearts develop focal areas of ischemic necrosis leading to dilated cardiomyopathy. Treadmill exercise produces massive coronary vasospasm, acute myocardial infarction, and sudden death. Remarkably, chronic verapamil treatment prevents all these cardiac abnormalities. But verapamil only works when used as a preventive measure in young mice. It does not reverse cardiac damage in the older mice.

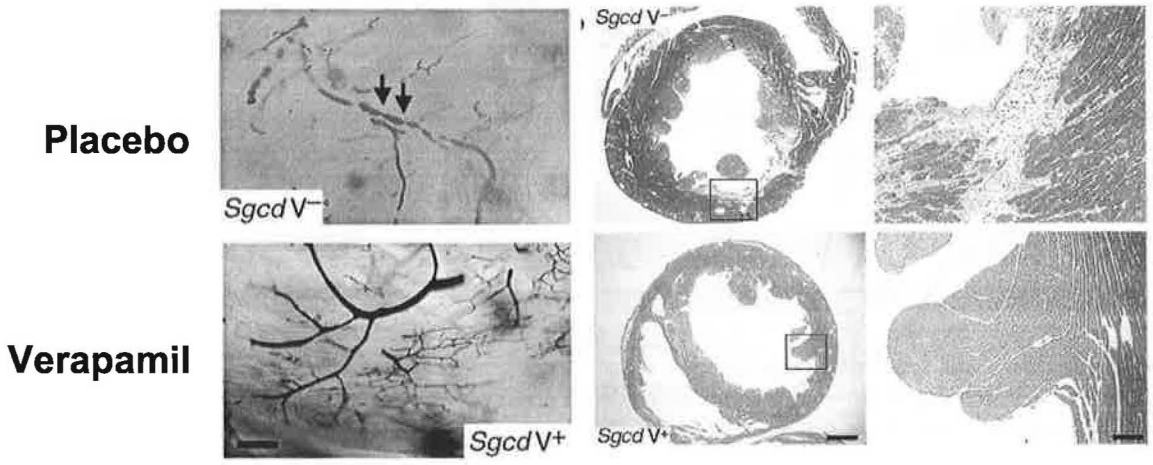

FIGURE 30. Prevention of coronary vasospasm and ischemic cardiomyopathy by verapamil in mice lacking the smooth muscle sarcoglycan-sarcospan complex. From RD Cohn et al., *JCI*, 2001.[29]

Exactly how sarcolygan deficiency results in vasospasm is unknown. NO deficiency may be involved because there is loss of sarcolemma nNOS from sarcoglycan-deficient muscle.[30]

Mice deficient in δ sarcoglycan provide an excellent experimental model for homozygous δ sarcoglycan deficiency in humans, LGMD2F. These patients develop severe skeletal muscle weakness and several cases of dilated cardiomyopathy have been described. In contrast, heterozygous δ sarcoglycan deficiency presents as familial or sporadic dilated cardiomyopathy in adolescents or young adults with completely normal skeletal muscle function.[31]

Lets now turn from these rare inherited diseases to consider dystrophin's role in the pathogenesis of common acquired forms of dilated cardiomyopathy…

VIRAL CARDIOMYOPATHY: AN ACQUIRED DYSTROPHINOPATHY

Coxsackie B viruses are known to be cardiomyopathic but, until recently, the mechanism of the cardiac injury was unknown. These viruses produce a protease (*protease 2A*) that normally cleaves the viral polyprotein into mature peptides. It turns out that protease 2A also cleaves dystrophin. The resultant disruption of the myocardial sarcolemma, in turn, makes it easier for additional viral particles to gain entry into the infected myocytes. Remarkably, the protease is inactivated by nitric oxide, which causes S-nitrosylation. Thus, NO donors such as nitroprusside prevent the protease from disrupting dystophin in vitro and limit the extent of myocardial damage in mice infected with Coxsackie B.

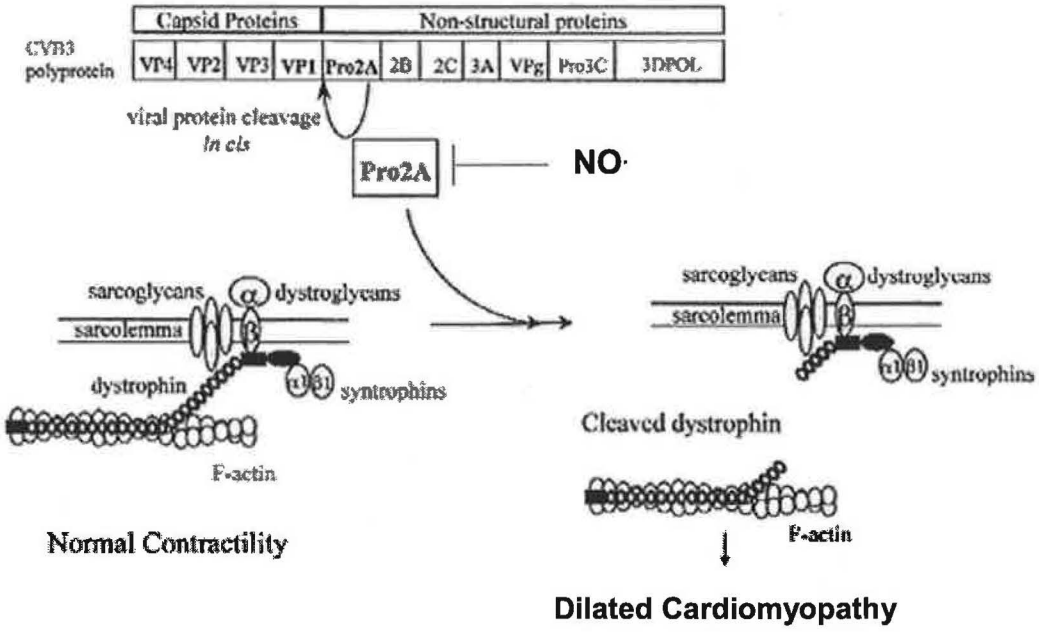

FIGURE 31. Dystrophin as a cellular target for enteroviral protease 2A.From C Badorff et al, *Nature Medicine*, 1999.[32]

The figure below shows the endomyocardial biopsy from the third case presentation, a 37 year-old woman with acute viral myocarditis.

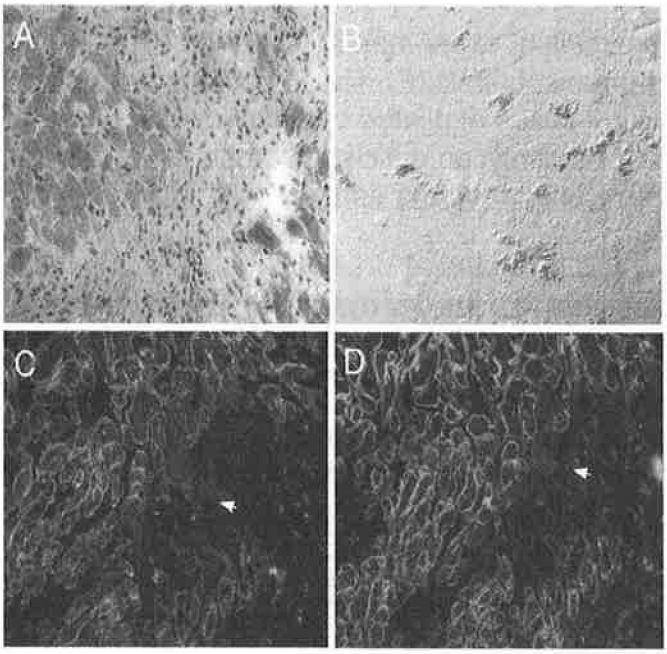

FIGURE 32. Ventricular biopsy from a patient with Coxsackie B myocarditis stained for:
A, hematoxylin and eosin
B, enteroviral (VPI) antigen
C, dystrophin
D, β-sarcoglycan

Arrows point to cardiac myocytes with disruption of the typical sarcolemmal localization of dystrophin and β-sarcoglycan. From C Badorff and KU Knowlton, *Med Microbiol Immunol*, 2004.[33]
Thus, enteroviral protease 2A is a putative new drug target for enteroviral cardiomyopathy.

Emerging data from cardiac magnetic resonance imaging indicate that acute viral myocarditis most commonly involves the left ventricular posterolateral wall, the same segment preferentially involved in DMD.

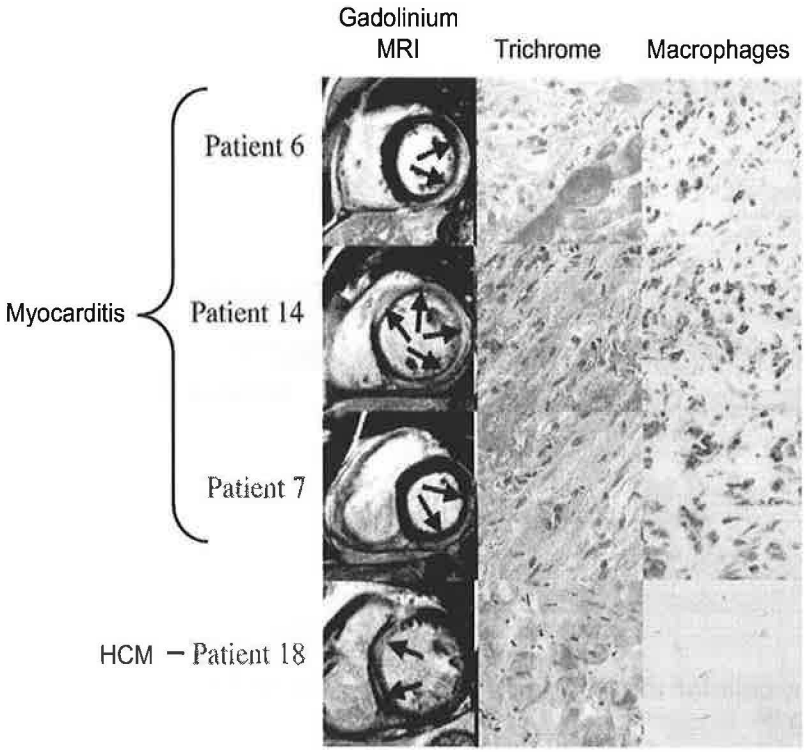

FIGURE 33. Cardiac MRI and histopathology of patients in whom biopsies were obtained from area of contrast enhancement. The top three patients had biopsy-proven myocarditis. The patient at the bottom had hypertrophic cardiomyopathy (HCM). Arrows point to white areas of contrast agent (gadolinium) enhancement, presumably due to its entry into cells with disrupted sarcolemmal membranes. From H Mahrholdt et al., *Circulation*, 2004.[34]

As in DMD, the explanation for the site-specificity of the cardiac pathology is unknown. But the parallelism is striking.

DYSTROPHIN CLEAVAGE AS A COMMON FINAL PATHWAY FOR HEART FAILURE PROGRESSION

In the mammalian heart, *calpain* is an endogenous calcium-activated protease that has been implicated to play a pivotal role in Ca^{2+} induced cell injury. Like enteroviral protease 2A, calpain also cleaves dystrophin.

In experimental and clinical heart failure, excessive stimulation of cardiac β-ARs by high circulating levels of catecholamines (epinephrine, norepinephrine) accelerate the deterioration of cardiac function in part by increasing flux of Ca^{2+} through voltage-gated channels resulting in activation of calpain which cleaves dystrophin. Experimental evidence to support this theory comes from three different experimental models: 1) infusion of isoproterenol (β-adrenergic agonist) to cause acute heart failure in normal rats, 2) progressive heart failure with aging in the Syrian hamster model of δ-sarcoglycan deficiency, and 3) acute myocardial infarction (coronary occlusion) in normal rats and dogs.

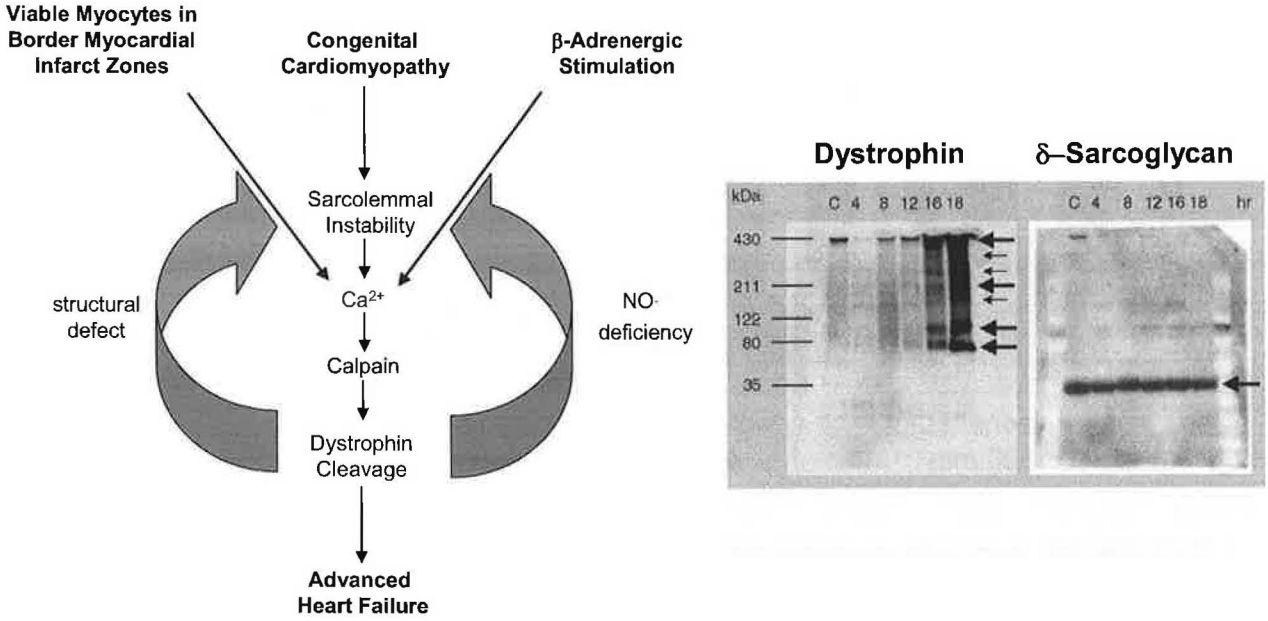

FIGURE 34. Dystrophin cleavage in multiple forms of heart disease. Left panel, dystrophin cleavage as a final common pathway for advanced heart failure; Right panel, Western blot experiments showing fragmentation of cardiac dystrophin in rats subjected to high-dose intravenous isoproterenol. From T Toyo-Oka et al., *PNAS*, 2004.[35]

Finally, myocardial dystrophin may be depleted in the common setting of acute coronary syndromes. In dog models of acute coronary occlusion and reperfusion, dystrophin is the first myocardial protein to be depleted from the infarct zones. This may be an important mechanism by which transient ischemia leads to permanent injury and eventually to ischemic cardiomyopathy.

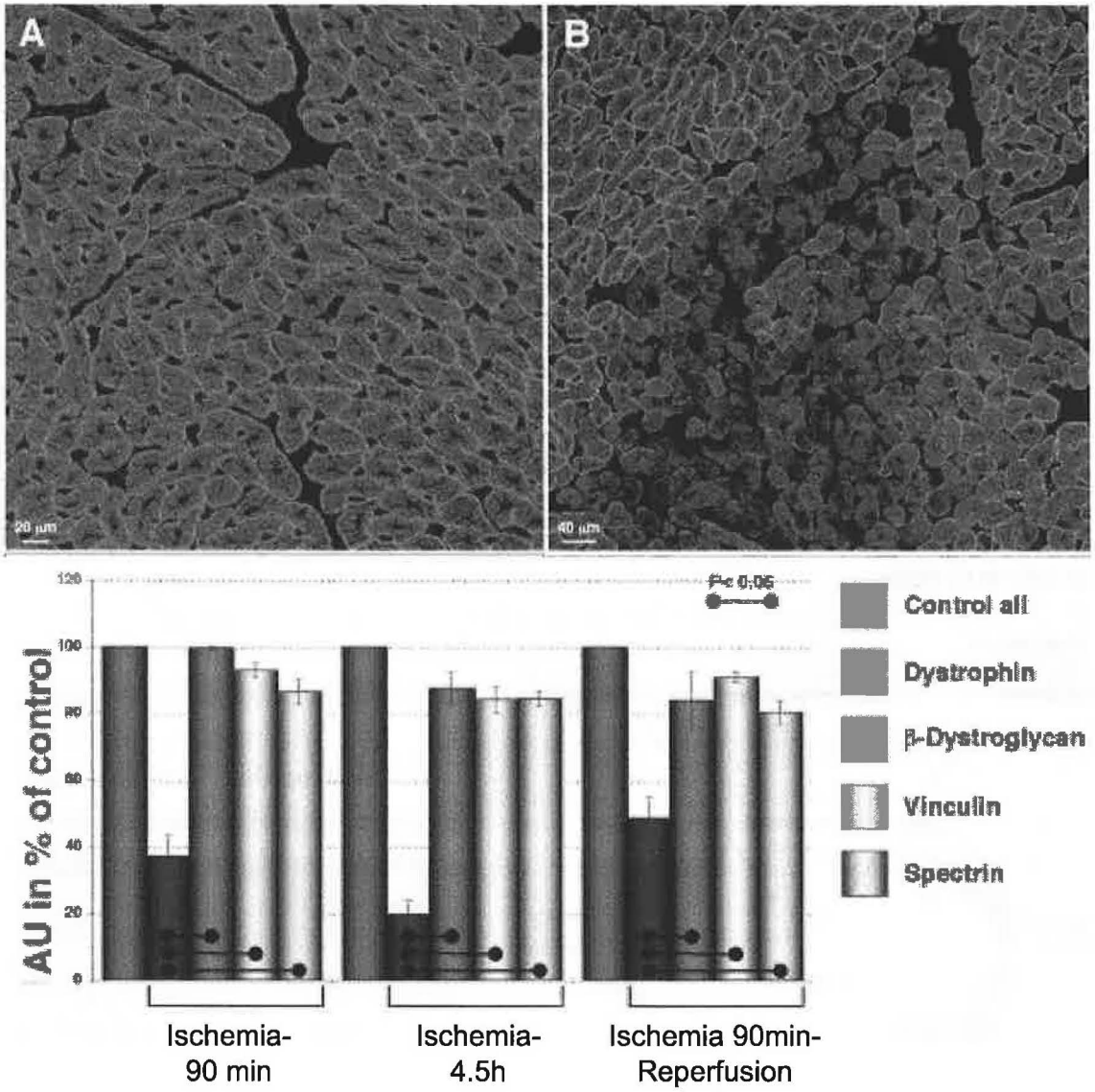

FIGURE 35. Ischemia depletes cardiac dystrophin. Top panel, uniform sarcolemmal (peripheral) pattern of dystrophin staining (green) in non-ischemic sections of myocardium (left) is replaced by a mosaic pattern in ischemic myocardium (right). Bottom panel, Greater ischemic sensitivity of dystrophin compared with other structural proteins. From M Rodriguez et al., *J Mol Cell Cardiol*, 2005.[36]

CONCLUSIONS

Inherited dystrophinopathy should be in the differential diagnosis of any patient with unexplained dilated cardiomyopathy, even in the absence of overt skeletal muscle disease. This is especially true in younger persons and those with a family history of cardiac or skeletal muscle disease. But by the time the patient presents with severe heart failure, such an etiologic diagnosis is strictly academic and has no impact on clinical decision making. Cardiac gene therapy is exciting but remains futuristic.

New genetic mouse models of the muscular dystrophies together with a much smaller number of translational human studies have made a new conceptual breakthrough with exciting therapeutic implications. Consistent results implicate a very important role for dystrophin-associated proteins (nNOS, sarcoglycans) in the normal regulation of vascular smooth muscle. Loss of such proteins produces microvascular ischemia that may accelerate the progressive destruction of structurally-defective skeletal and cardiac muscle in certain forms of inherited and acquired dystrophinopathies.

Thus, Duchenne muscular dystrophy and other dystrophinopathies may constitute a new indication for some old cardiovascular drugs: direct vasodilators, calcium channel blockers and central sympatholytics. Because dilated cardiomyopathy is a late manifestation of dystrophinopathy, early treatment of functional muscle ischemia in children with DMD, Becker's muscular dystrophy as well as female carriers and those with LGMD may extend life by preventing or at least delaying dilated cardiomyopathy. This is a fertile area for future multi-disciplinary research.

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