

PW 3:
**Treatment approaches to
the mdx mouse model**

PW3-029	<p><u>MUSCLE-SPECIFIC DIFFERENCES IN FIBROSIS AND TGFβ-1 AND MMP-9 EXPRESSION IN NONDYSTROPHIC AND MDX MICE.</u> GRAHAM K¹, SINGH R¹, MILLMAN G¹, MALNASSY G¹, BERGE J¹, GATTI F¹, CARLSON CG¹ (1) AT Still University, Kirksville, USA.</p>
To contact the author:: ccarlson@atsu.edu.	<p>Hydroxyproline assays were used to assess developmental changes in fibrosis in mdx and nondystrophic mice. In both adult nondystrophic and mdx mice, hydroxyproline levels (μg hydroxyproline/mg wet weight) were significantly higher in the costal diaphragm than in the gastrocnemius. The expression of TGFβ and MMP-9 were also significantly higher in the costal diaphragm than in the gastrocnemius of adult nondystrophic and mdx mice. In comparison to age-matched nondystrophic mice, adult mdx mice did not exhibit elevated hydroxyproline levels in the gastrocnemius, but did exhibit substantially elevated levels in the costal diaphragm. TGFβ expression was also significantly higher in mdx gastrocnemius and costal diaphragm than in the corresponding nondystrophic muscle preparations. In contrast, expression of MMP-9 was virtually identical in nondystrophic and mdx costal diaphragm, but was significantly higher in the mdx gastrocnemius than in the corresponding nondystrophic preparation. These results suggest that hydroxyproline levels are substantially and significantly higher in the adult mdx costal diaphragm due to increases in the expression of TGFβ that are not balanced by corresponding increases in the expression of matrix metalloproteases. Examination of hydroxyproline levels in nondystrophic and mdx triangularis sterni (TS) muscle and the costal and crural diaphragm indicated that the respiratory musculature of both nondystrophic and mdx mice exhibit muscle-specific differences in hydroxyproline expression with the highest hydroxyproline levels observed in the costal diaphragm. Relative to age-matched nondystrophic mice, significant increases in hydroxyproline were first observed in the mdx TS muscle at 1 month of age and in the costal diaphragm at 2 months of age. The largest proportional increase in hydroxyproline over nondystrophic levels (13.5 fold increase) was observed in the mdx TS at 4 months of age. These results indicate that the signaling pathways responsible for collagen deposition are muscle-specific and depend upon a balance between fibrogenic and fibrolytic factors. (Supported by AFM, Charley's Fund, Strategic Research Grant from ATSU)</p>

PW3-030	<p><u>THE SEVERELY DYSTROPHIC MDX TRIANGULARIS STERNI (TS) MUSCLE IS A USEFUL PREPARATION FOR EXAMINING DRUG EFFICACY IN THE MDX MOUSE MODEL.</u></p> <p> SIEGEL A¹, ZIMMERMAN A¹, HENLEY S¹, RHODES J¹, MILES M¹, SHIN G², BECK B¹, HOFF H¹, KURZ J¹, BALCH F¹, CARLSON CG¹ (1) AT Still University, Kirksville, USA. (2) Truman State University, Kirksville, USA. </p>
To contact the author:: ccarlson@atsu.edu.	<p>The TS is an expiratory muscle that is passively stretched with each inspiration and concentrically activated with each expiration. The degree of passive stretch of TS muscle fibers depends upon the location of the fiber within the muscle, with caudal fibers stretched up to 10% more than cephalad fibers (DeTroyer et al., J. Physiol. (Lond.) 513.3, 915-925, 1998). Cross sections of adult nondystrophic TS indicate roughly uniform fiber density in the caudal, middle, and cephalad thirds of the muscle and fiber diameters that increase significantly in the cephalad to caudal direction. In comparison to age-matched nondystrophic mice, the density of muscle fibers in mdx TS muscles at 1 to 2 years of age was significantly reduced in the middle and cephalad regions of the TS muscle, and the cross-sectional diameter significantly reduced in all 3 regions. The total working diameter and working cross sectional area of mdx TS muscle was also significantly reduced relative to nondystrophic values. Increased centronucleation was observed in all 3 regions of the mdx TS muscle. Transmission electron micrographs from mdx TS indicate substantial Z line streaming, sarcomere disorganization, and numerous hypercontracted areas where dense myofilamentous material was observed adjacent to empty sarcoplasm containing numerous mitochondria. Long term treatment of mature adult mdx mice with pyrrolidine dithiocarbamate (PDTC; 50 mg/kg, intraperitoneal) produced a significant increase in fiber diameter in the middle TS and reduced centronucleation throughout the TS. Daily treatment with N-acetylcysteine (NAC; 100 mg/kg, ip) for a period of 3.5 months also significantly increased the fiber diameter and fiber cross-sectional area in the cephalad region of the mdx TS. These results provide evidence supporting the utility of the mdx TS preparation in assessing the morphological benefits of a variety of potential treatments for Duchenne and related muscular dystrophies. (Supported by AFM, Charley's Fund, Strategic Research Grant from ATSU)</p>

PW3-031	<p><u>DIRECT EFFECT OF PYRROLIDINE DITHIOCARBAMATE ON THE RESTING MEMBRANE POTENTIAL OF FRESHLY ISOLATED MDX MUSCLE FIBERS.</u></p> <p>MILES M¹, CARLSON CG¹ (1) AT Still University, Kirksville, USA.</p>
To contact the author:: ccarlson@atsu.edu.	<p>Daily treatment of adult mdx mice with intraperitoneal injections of pyrrolidine dithiocarbamate (PDTC) substantially improved the resting membrane potential in severely dystrophic (mdx) triangularis sterni (TS) muscle fibers (Carlson et al., <u>Neurobiology of Disease</u> , 20 (3), 719-730, 2005). To determine whether this effect was mediated by circulating factors exogenous to skeletal muscle, the effect of PDTC on the resting potential of freshly excised triangularis sterni (TS) muscle fibers was examined <u>in vitro</u>. Although PDTC had no effect on the resting potential of nondystrophic fibers, 100 μM PDTC produced a significant 6.9 mV increase in the average resting potential of mdx TS fibers (7.5 month, caudal region) over a 90 minute interval. Pre-treatment with ouabain abolished this restorative effect of PDTC on the resting potential of mdx fibers. To determine the contribution of the Na⁺ - K⁺ ATPase on the resting membrane potential, nondystrophic and mdx fibers were exposed to 10⁻³ M ouabain for 30 minutes. The reduction in resting potential produced by ouabain in mdx TS fibers (Δ4.3 mV) was significantly and substantially less than that observed in nondystrophic TS fibers (Δ14.7 mV). Fluorimetric determinations (F340/F380) in SBFI loaded muscle fibers also suggested that intracellular Na⁺ is elevated in mdx TS fibers. Consistent with the resting potential measurements, the increase in F340/F380 induced by ouabain in nondystrophic fibers was substantially and significantly greater than that observed in mdx fibers. These results suggest that: (1) the reduced resting potentials in dystrophic muscle are associated with altered Na⁺-K⁺ pump activity, increases in intracellular Na⁺, and a reduction in the outward Na⁺ pump current; and (2) PDTC improves the resting potential by a direct or indirect effect on Na⁺-K⁺ pump activity. (Supported by AFM, Charley's Fund, Strategic Research Grant from ATSU)</p>

PW3-032	<p><u>EFFECTS OF TREATMENT WITH URSODEOXYCHOLIC ACID (UDCA) ON P65 NUCLEAR ACTIVATION AND WHOLE BODY TENSION (WBT) DEVELOPMENT IN THE MDX MOUSE</u></p> <p>TURIN E¹, HOFF H¹, GATTI F¹, WINDERS T¹, SINGH R¹, STARKE J¹, RUTTER J¹, BLEDSOE C¹, LAVIN J¹, PALMIERI B², CARLSON CG¹ (1) AT Still University, Kirksville, USA. (2) University of Modena, Modena, ITALY.</p>
To contact the author:: ccarlson@atsu.edu.	<p>Ursodeoxycholic acid (UDCA) is in current clinical use for the treatment of biliary cirrhosis and has been shown to reduce nuclear p65 activation in HeLa cells expressing elevated glucocorticoid receptor (Miura et al., J. Biol. Chem., 276(50), 47371-47378, 2001). Nuclear extracts obtained from mdx diaphragms exposed to 100 or 200 μM UDCA for 2 hours exhibited a significant 65% reduction in p65 activation in comparison to mdx preparations bathed in HEPES Ringer for the same period. Preparations exposed to 200 μM UDCA for 4 hours also exhibited significant reductions in nuclear p65 activation to approximately 50% of the levels seen in preparations exposed to HEPES Ringer. To examine the efficacy of UDCA administration, several adult mdx mice were administered a single dose of 5, 10, or 20 mg/kg intraperitoneally and euthanized at 3 and 5 hours after injection (vehicle treated mdx served as controls). UDCA exposure did not reduce nuclear p65 activation at 3 hours after the injection but significantly reduced activation at all 3 doses to approximately 33% of vehicle-treated levels at 5 hours after the injection. Daily treatment of 1 month old mdx mice at 40 mg/kg for a period of 30 days significantly reduced nuclear p65 activation to approximately 60% of vehicle-treated levels. Whole body tension determinations obtained after 30 days of UDCA treatment indicated significant, 24 to 27%, increases in the WBT5 and WBT10 measures (Carlson and Makiejus, Muscle and Nerve, 13:480-484, 1990) corresponding to 31 and 26% recovery in WBT5 and WBT10, respectively. UDCA treatment had no effect on the WBT10/WBT5 ratio or on 4 limb hang time using a wire grid. These experiments suggest that UDCA may be effective in the treatment of Duchenne and related muscular dystrophies. Experiments examining the efficacy of oral administration of UDCA in the mdx mouse are underway. (Supported by AFM, Charley's Fund, Strategic Research Grant from ATSU)</p>

PW3-033	<p><u>INCREASES IN THE EXPRESSION OF ALTERNATIVE NFKAPPA-B PATHWAY SIGNALING COMPONENTS IN ADULT MDX SKELETAL MUSCLE</u> SINGH R¹, SAMADI A², CARLSON CG¹ (1) AT Still University, Kirksville, USA. (2) University of Kansas Medical Center, Kansas City, USA.</p>
To contact the author:: ccarlson@atsu.edu.	<p>Although several reports indicate that dystrophic muscle exhibits elevated nuclear p65 activation, little is known regarding the disposition of the alternative pathway in dystrophic muscle. In nondystrophic muscle, several of the components of the alternative NF-κB signaling pathway are upregulated by disuse and this upregulation is associated with a reduction in the expression of pro-apoptotic proteins and an increase in expression of anti-apoptotic proteins (Hunter et al., FASEB J., 16, 529-538, 2002). To examine the potential role of the alternative NF-κB pathway in muscular dystrophy, cytosolic, nuclear, and whole cell extracts from adult nondystrophic and mdx costal diaphragms were used to examine the expression of NF-κB signaling components along the alternative pathway. Whole cell extracts obtained from mdx costal diaphragm exhibited significantly increased expression of rel B, p 52, and p100 (Western blot densitometric analysis with GAPDH as loading control) in comparison to nondystrophic diaphragm. The ratio of p52/p100 which represents the activity of the proteasome in degrading p100 was also significantly increased in dystrophic muscle. These increases were associated with corresponding increases in the expression of IKKα and in the phosphorylation of NIK (P_i NIK/total NIK). These results provide evidence that both the classical and alternative NF-κB pathways exhibit elevated signaling in adult dystrophic skeletal muscle, and indicate a need for additional studies to identify whether the alternative pathway is compensatory or detrimental to the structure and function of dystrophic skeletal muscle. (Supported by AFM, Charley's Fund, Strategic Research Grant from ATSU)</p>

PW3-034	<p><u>INCREASES IN THE EXPRESSION OF CLASSICAL NFKAPPAB SIGNALING COMPONENTS IN ADULT MDX SKELETAL MUSCLE.</u> SINGH R¹, MILLMAN G¹, POLISIAKEIWICZ L¹, TURIN E¹, SUMSKI C¹, SAMADI A², CARLSON CG¹ (1) AT Still University, Kirksville, USA. (2) University of Kansas Medical Center, Kansas City, USA.</p>
To contact the author:: ccarlson@atsu.edu.	<p>The development of more efficacious treatments for Duchenne and related muscular dystrophies would be facilitated by an improved understanding of the mechanism which promotes enhanced nuclear p65 activation in dystrophic skeletal muscle. To accomplish this objective, the status of the classical pathway was examined in cytosolic, nuclear, and whole cell extracts from adult nondystrophic and mdx costal diaphragm. Although absolute p65 activation was increased several fold in nuclear extracts from mdx diaphragm (Trans AM ELISA based assay), the proportion of activated p65 in the nuclear compartment (nuclear/nuclear + cytosolic) was identical in nondystrophic and mdx muscle preparations. Consistent with this observation, cytosolic and whole cell densitometric levels (GAPDH loading control) of IκB-α were not decreased, but were significantly increased in the mdx diaphragm. The proportion of cytosolic phosphorylated IκB-α (P_r- IκB-α/ total IκB-α) was the same in nondystrophic and mdx diaphragm. Whole cell extracts from mdx muscle exhibited significant increases in the expression of both p65 and p50, and increases in the proportion of phosphorylated p65 (P_r- p65/ total p65). The expression of IκB kinase (IKK) α, β, and γ, the proportion of phosphorylated IKKα and the expression of SUMO-1 were also significantly increased in mdx muscle. To examine the potential role of the proteasome in regulating cytosolic IκB-α, preparations were exposed to the inhibitor MG132. This treatment to decrease IκB-α degradation significantly increased cytosolic IκB-α levels in nondystrophic, but had no significant effects on cytosolic IκB-α in mdx diaphragm. These results indicate that baseline reductions in cytosolic IκB-α do not occur in dystrophic skeletal muscle, and that increases in the expression of each of the signaling components of the classical NFκB pathway contribute to the increases in nuclear p65 activation. (Supported by AFM, Charley's Fund, Strategic Research Grant from ATSU)</p>

PW3-035	<p><u>NAV1.4 DEREGULATION IN DYSTROPHIC SKELETAL MUSCLE LEADS TO Na⁺ OVERLOAD AND ENHANCED CELL DEATH</u> HIRN C¹, SHAPOVALOV G¹, ROULET E¹ (1) Laboratory of pharmacology, Geneva, SWITZERLAND.</p>
To contact the author:: emmanuelle.roulet@pharm.unige.ch.	<p>Duchenne Muscular dystrophy (DMD) is manifested by the absence of dystrophin – a structural, cytoskeletal protein – leading to muscle degeneration. Whereas the rise of cytosolic Ca²⁺ concentration has been extensively documented in the muscle of <i>mdx</i> mouse, little is known about alterations of Na⁺ concentration. We observed that ²²Na⁺ influx is elevated in <i>mdx</i> FDB muscle and tetrodotoxin (TTX 3nM) an inhibitor of voltage-gated sodium channels restored Na⁺ influx in <i>mdx</i> to that of control fibers without affecting the latter. Here we show, for the first time, that the skeletal isoform of VGSC, namely Na_v1.4, which represents over 90% of VGSCs in muscle, is responsible for abnormal Na⁺ concentrations found in muscle from <i>mdx</i> mice. The absence of dystrophin modifies the expression level and gating properties of Na_v1.4 leading to an increased level of [Na⁺] under the sarcolemma. Indeed, we observed a reduced inactivation rate and a reduction of the reversal potential for Na⁺ by 18 mV, indicating an about 2-fold higher [Na⁺] under sarcolemma in <i>mdx</i> fibres as compared to those of control. Moreover we observed that Na_v1.4 colocalized with α1-syntrophins belonging to dystrophin-associated protein complex and the distribution of both proteins was altered in dystrophin-deficient fibers. To supplement these findings, we studied the survival of <i>mdx</i> and control fibers in culture and found an approximately 30% higher mortality rate of dystrophic fibers. Na_v1.4 inhibition with tetrodotoxin improved survival of <i>mdx</i> isolated fibers in culture close to the one of control fibers over a period of 3 days. These results suggest that modifications of Na_v1.4 properties and excess Na⁺ strongly correlate with increased cell death in <i>mdx</i> fibres.</p>

PW3-036	<p><u>ROLE OF TRPC1 AN TRPC3 IN THE EXCESS OF CA2+ CONCENTRATION OBSERVED IN DUCHENNE MUSCULAR DYSTROPHY</u> DHAMANE E¹, GALLO C¹, PETERMANN O¹, RUEGG U¹, ROULET E¹ (1) Laboratory of Pharmacology, section of Pharmaceutical Sciences, University of Geneva, Geneva, SWITZERLAND.</p>
To contact the author:: emmanuelle.roulet@pharm.ubige.ch.	<p>Calcium dysbalance is expected to be one of the triggering events causing muscular degeneration in Duchenne muscular dystrophy (DMD). It has been proposed that the increased Ca²⁺ influx could result from transient membrane lesions (Menke <i>et al.</i>, 1995; Petrof <i>et al.</i>, 1993) or from influx through plasma membrane Ca²⁺ channels. Recent results indicate that the increased permeability of the sarcolemma of dystrophic fibres may be due to increased activity of cationic channels belonging to the TRP family which could result in an increase of Ca²⁺ concentration. Both, stretch-activated channels (SAC) and store-operated channels (SOC) have been proposed to be involved in the enhanced Ca²⁺ entry occurring in dystrophic skeletal muscle cell. In our study we focused mainly on TRPC family. We and others have shown that TRPC1 as well as TRPC 2,3,4 and 6 are expressed in skeletal muscle and were proposed to be candidates for these Ca²⁺ channels (Vandebrouck <i>et al.</i>, 2002). In a series of experiments we have shown by quantitative RT-PCR that TRPC3 mRNA is overexpressed in <i>mdx</i> muscles compared to wt. This prompted us to consider TRPC3 as a potential candidate for the increase of Ca²⁺ influx described in <i>mdx</i> muscle. TRPC1 has been described as important and necessary for (SOC) and (SAC). Therefore we have cloned a series of shRNAs directed against TRPC1 an TRPC3 into the adeno-associated viral vector type 2 and 6 (pAAV) together with the Pol III promoter H1. We have validated the efficiency of our constructions in heterologous systems and we have obtained an inhibition of TRPC3 and TRPC1 mRNAs from 50% up to 90%. We are currently producing high titer viruses in order to transduce our myotubes from EDL-<i>mdx</i> cell line as well as <i>mdx</i> FDB fibres and we will analyse the effect of their inhibition on calcium</p>

PW3-037	<p><u>BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) REGULATES SKELETAL MUSCLE REGENERATION AND IS MIS-REGULATED IN DYSTROPHIC MUSCLE</u> CLOW C[†], MOUSAVI K[†], RAVEL-CHAPUIS A[†], JASMIN B[†] (1) University of Ottawa, Center for Neuromuscular Disease, Ottawa, CANADA.</p>
To contact the author:: cclow072@uottawa.ca.	<p>Muscle-derived BDNF has long been thought to serve as a retrograde trophic factor for innervating motor neurons throughout their lifespan. However, our recent studies have shown that BDNF is not enriched at the neuromuscular junction in adult skeletal muscle, and instead, is highly expressed in satellite cells (J. Neurosci., 26: 5739, 2006). Furthermore, we have shown that BDNF depletion results in precocious differentiation of myoblasts in culture. These findings suggest a role for BDNF in myogenic differentiation. In order to expand on these findings, and determine whether BDNF plays a similar role in vivo, we designed a series of complementary experiments to elucidate the role of BDNF in muscle development, regeneration and disease. First, we generated a mouse in which BDNF is specifically depleted in skeletal muscle. Characterization of the muscle-specific BDNF knockout mouse showed abnormal neuromuscular junction formation, and fiber-type switching. Next, we compared regeneration of wild-type and BDNF-depleted muscle following cardiotoxin injury. BDNF-depleted muscle showed delayed regeneration, as indicated by delayed appearance of centrally nucleated fibers and developmental myosin heavy chains (MyHC). Finally, given the therapeutic importance of regeneration for treating various muscle diseases, we examined the expression profile of BDNF in dystrophic muscles from mdx mice. Compared to wild-type, aged mdx muscles consistently displayed greater levels of BDNF. Interestingly, high levels of BDNF correlate with the age at which regeneration becomes defective in mdx muscle, resulting in rapid deterioration and early death compared to wild-type. These findings demonstrate that muscle-derived BDNF is important for regulating: i) the organization of the post-synaptic compartment of muscle fibers; ii) the pattern of MyHC isoform expressed; and iii) the regenerative potential of muscle. Based on these findings, BDNF manipulation may represent an important therapeutic tool to alleviate the dystrophic muscle pathology.</p> <p>This work is supported by CIHR, AFM and MDA (USA).</p>

PW3-038

SUBCUTANEOUS INJECTION FROM BIRTH OF EPIGALLOCATECHIN-3-GALLATE, A COMPONENT OF GREEN TEA, LIMITS THE ONSET OF MUSCULAR DYSTROPHY IN MDX MICE: QUANTITATIVE HISTOLOGICAL AND ELECTROPHYSIOLOGICAL STUDY

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<p>To contact the author:: yoshiko.nakae@pharm.u nige.ch.</p>	<p>Dystrophic muscles suffer from enhanced oxidative stress. We have investigated whether administration of an antioxidant, epigallocatechin-3-gallate (EGCG), a major polyphenol of green tea, reduces their oxidative stress and pathophysiology in <i>mdx</i> mice, a mild phenotype model of human Duchenne-type muscular dystrophy. EGCG (5mg/kg body weight in saline) was injected subcutaneously four times a week into the backs of C57BL/10 normal mice and dystrophin-deficient <i>mdx</i> mice for 8 weeks from either the day of birth or a day after birth. Saline was injected into normal and <i>mdx</i> controls. At the end of the treatment EGCG had almost no observable effects on normal mice or on the body weights of <i>mdx</i> mice. In contrast, it produced the following improvements in the blood chemistry, muscle histology and electrophysiology of the treated <i>mdx</i> mice. First, the activities of serum creatine kinase, an index of muscle damage, were reduced to near normal levels. Second, the numbers per unit volume of an oxidative stress marker, autofluorescent lipofuscin granules, in soleus and diaphragm muscles were significantly decreased by about 50 % compared to the numbers in the corresponding saline-treated controls. Third, in sections of diaphragm muscles, the relative area of histologically normal muscle fibres increased significantly about 2-fold whereas the relative areas of connective tissue and necrotic muscle fibres were significantly reduced by about 40 and 90% respectively. In sections of soleus muscles the relative area of normal muscle fibres significantly increased about 1.5-fold but that of necrotic muscle fibres decreased by 95 %. Fourth, the times for the maximum tetanic force of soleus muscles to fall by a half increased to almost normal values. Our study corroborates other recent studies that EGCG is effective for limiting the onset of muscular dystrophy in <i>mdx</i> mice without causing side effects.</p>
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<p>PW3-039</p>	<p><u>INCREASED DENSITY OF INHIBITORY SYNAPSES AND SIZE OF PERFORATED-EXCITATORY SYNAPSES IN CA1 HIPPOCAMPUS OF DYSTROPHIN-DEFICIENT MDX MICE: A VOLUMETRIC MRI AND SERIAL ELECTRON MICROSCOPY STUDY</u> MIRANDA R⁴, SEBRIÉ C², DEGROUARD J³, JAILLARD D³, LAROCHE S¹, VAILLEND C¹ (1) Laboratoire de Neurobiologie de la Mémoire, de l'Apprentissage et de la Communication, CNRS UMR 8620, Université Paris-Sud, Orsay, FRANCE. (2) Laboratoire de RMN Biologique, ICSN-CNRS, Gif sur Yvette, FINLAND. (3) Centre Commun de Microscopie Electronique (CCME), UMR 8080 CNRS, Université Paris-Sud, Orsay, FRANCE. (4) Biological Psychology Area, University of the Balearic Islands, Palma de Mallorca, SPAIN.</p>
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Mdx mice provide a model of the human X-linked muscular dystrophy (DMD) caused by mutations in the DMD gene inducing absence of 427-KDa cytoskeletal protein dystrophin. Not only expressed in muscle fibers, dystrophin is also present in the postsynaptic densities (PSDs) of pyramidal cells in cerebral cortex, hippocampus and cerebellum, which may explain the association of DMD with various degrees of cognitive alterations. In the hippocampus, dystrophin is found in perisomatic area and proximal dendrites of CA1-4 pyramidal neurons. In mdx mice, the loss of dystrophin is associated with reduced number and size of GABA_A-receptor clusters and abnormal facilitation of CA1 hippocampal long-term potentiation (LTP), suggesting that the memory deficits observed in this model could rely on altered synaptic inhibition and/or imbalanced excitation/inhibition (E/I). The neuronal bases of these alterations, however, have not yet been investigated in detail. Here, we used MRI, light and electron microscopy approaches to investigate possible changes in hippocampal volume, neuron number, and synapse ultrastructure in mdx mice. First we show that dystrophin loss does not affect whole-brain or hippocampal volumes, or the CA1 pyramidal cell density and total synapse number in both the proximal and distal dendritic layers of the CA1 hippocampal subfield. However, we found that the number of axodendritic-inhibitory synapses is enhanced in the proximal dendritic layer of CA1 in mdx mice, a region that normally shows selective expression of dystrophin in association with GABA_A receptors. Furthermore, the length of the PSDs is abnormally increased in axospinous perforated-excitatory synapses of mdx mice. Our results suggest that compensatory mechanisms occur during the formation of new inhibitory synapses to counteract a reduced capacity for GABA_A-receptor clustering, and we hypothesize that the GABAergic alterations due to dystrophin loss from inhibitory synapses also affect the functional plasticity of glutamatergic synapses.

PW3-040	<p><u>PLASMIN(OGEN) ACTIVITY IS REQUIRED DURING MYOGENESIS IN VITRO AND MUSCLE REGENERATION IN VIVO IN AN ALPHA-ENOLASE-DEPENDENT MANNER.</u></p> <p>LLORENS A¹, DIAZ-RAMOS MA¹, LOPEZ-ALEMANY R¹ (1) IDIBELL, L'Hospitalet de Llobregat, SPAIN.</p>
To contact the author:: allorems@idibell.org.	<p>The Plasminogen Activation (PA) system is a group of serin-proteases that plays an important role in a wide range of biological processes in which tissue remodelling takes place. Plasmin, generated by activation of its zymogen plasminogen, is a potent protease able to degradate most of the extracelllular matrix components. Alpha-enolase constitutes a receptor for plasmin(ogen) in several cell types, where it acts focalizing proteolytic activity on the cell surface.</p> <p>Previous studies have shown a role for urokinase-type PA and plasminogen during myogenesis <i>in vitro</i> and <i>in vivo</i>. We have previously shown that alpha-enolase expression was up-regulated in murine myoblasts cell line, C2C12, upon differentiation, paralleling plasmin activity on the cell surface. The role of alpha-enolase as a plasminogen receptor in myogenesis deserves further analysis.</p> <p>To analyze the role of alpha-enolase in myogenesis <i>in vitro</i>, we have used different inhibitors: a monoclonal antibody against alpha-enolase, MAb11G1, and a lysine analogue, EACA, both blocking alpha-enolase/plasminogen binding. Using primary cultures of myogenic cells or <i>Muscle Precursor Cells</i> (MPCs), both inhibitors abrogated myogenic differentiation, fusion and migration. In contrast, plasmin activity inhibitors, as aprotinin, had no effect.</p> <p>The effect of inhibitors was also evaluated in a regeneration model in mice after an injury. Regeneration parameters were diminished by of MAb11G1 and EACA, as well as inflammatory cells infiltration and increased fibrin deposition. When we injected <i>mdx</i> mice (the animal model for Duchenne Muscular Dystrophy), the plasminogen/alpha-enolase binding inhibitors presented a more severe dystrophinopathy than control mice.</p> <p>Since inhibitors of plasminogen/alpha-enolase binding have an inhibitory effect on MPCs differentiation <i>in vitro</i> and muscle regeneration <i>in vivo</i>, our results demonstrate by the first time that plasmin activity is necessary for myogenesis to take place correctly, in an alpha-enolase dependent way. Plasminogen/alpha-enolase binding therefore could be an important target in the development of treatments for DMD.</p>

PW3-041	<p><u>THE DECREASE OF EXPRESSION OF RYANODINE RECEPTOR SUBTYPE 2 IS REVERSED BY GENTAMYCIN SULFATE IN VASCULAR MYOCYTES FROM MDX MOUSE.</u></p> <p>MOREL JL¹, FRITZ N², DABERTRAND F¹, MACREZ N¹, HENAFF M¹, MIRONNEAU J³, MIRONNEAU C³</p> <p>(1) umr5228 CNRS universit� Bordeaux, talence, FRANCE. (2) karolinska institut, molecular neurobiology, stockholm, SWEDEN. (3) retired umr5017 CNRS universit� de bordeaux, Bordeaux, FRANCE.</p>
To contact the author:: jl.morel@cnic.u-bordeaux1.fr.	<p>The mdx mouse, a model of the human Duchenne muscular dystrophy displays incompletely understood impaired contractile function of skeletal, cardiac and smooth muscles. We explored the possibility that ryanodine receptor (RYR) expression could be altered in vascular muscle. The three RYR subtypes are expressed in portal vein myocytes. As observed on the mRNA and protein levels, RYR2 expression was strongly decreased in mdx myocytes whereas RYR3 and RYR1 expression were unaltered. The use of antisense oligonucleotide directed against RYR subtypes indicated that caffeine-induced Ca²⁺ response depended on RYR1 and RYR2. In mdx mouse, caffeine-induced Ca²⁺ responses was decreased in both amplitude and maximal rate of rise and the frequency of Ca²⁺ sparks was also strongly decreased. The gentamycin treatment of mdx mice was able to restore both the expression of RYR2 and the caffeine-induced Ca²⁺ response at the same level that observed in wild-type mice. Taken together, these results confirm that both RYR1 and RYR2 are required for vascular Ca²⁺ signalling and indicate that inhibition of RYR2 expression may account for the decreased Ca²⁺ release from the SR in mdx vascular myocytes. These results may help to explain the reduced efficacy of contraction in vascular myocytes of mdx mice and possibly Duchenne muscular dystrophy-afflicted patients. Finally, we suggest that gentamycin treatment of mdx mice could restore the Ca²⁺ signalling in smooth muscle and possibly the vascular function.</p>

PW3-042	<p><u>NEW INSIGHT ON THE BENEFICIAL EFFECT OF L-ARGININE ON MDX MUSCLE: IMPACT ON INFLAMMATION, CALCIUM HOMEOSTASIS AND SARCOLEMMA INTEGRITY.</u></p> <p>HNIA K¹, GAYRAUD G¹, LACAMPAGNE A², KOECHLIN C³, HUGON G¹, RIVIER F¹, DE LA PORTE S⁴, MORNET D¹, MATECKI S¹</p> <p>(1) INSERM ERI 25, Montpellier, FRANCE. (2) INSERM U 637, Montpellier, FRANCE. (3) INRA remodelage musculaire, Montpellier, FRANCE. (4) CNRS UPR 9040, Gif sur Yvette, FRANCE.</p>
To contact the author:: s-matecki@chu-montpellier.fr.	<p>L-Arginine was proposed as a potential pharmacological tool in Duchenne muscular dystrophy (DMD), a progressive-muscle wasting disease due to mutations in the dystrophin gene. Despite the beneficial effect on L-arginine on muscle weakness and force, mechanisms by each this molecule acts on muscle remain poorly investigated. Here, we showed that L-Arginine administration to 5 week-old mdx mice improves Ca²⁺-sparks's properties with a significant increase in spark amplitude, a shorter rise-time and increase spatial spread. In the other hand we demonstrate that L-arginine treatment decreases inflammatory secreted cytokine IL-1α, IL-6 and TNF-α targeted to dystrophic muscle fibres. This leads to decrease level and activity of NF-kB and its targeted proteins such as the muscle specific metalloproteinases MMP-2 and MMP-9. The β-dystroglycan (a key transmembrane glycoprotein of the dystrophin glycoprotein complex) which anchors utrophin to sarcolemma is a target protein of the MMP-2 and MMP-9 in mdx muscle. Increase activity of MMPs promotes an abnormal cleavage of the β-dystroglycan into a ~30 kDa form. Acting on NF-kB/MMPs cascade L-arginine promotes a better membrane stability of β-dystroglycan/utrophin couple and re-localizes nNOS in subsarcolemmal compartment of the dystrophic fibres which could explain the beneficial effect on resistance to contraction-induced mechanical stress observed in treated muscle.</p> <p>In conclusion our study points on the involved signaling cascades targeted by L-arginine in dystrophin-deficient muscle and strengthens the use of L-arginine as a potential pharmacological tool in Duchenne muscular dystrophies and also enlarges its use in other muscle-inflammation mediated myopathies.</p>