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【Keynote lecture II】

**Seeing muscle damage and regeneration from the inside out- Effects of Dystrophin, Transmembrane proteins, ECM proteins in skeletal muscle**

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**Abstract:**

The purpose of this study is to investigate the change of the maximum Contractile force, breaking load force and the contents of cytoskeleton protein of the gastrocnemius affected by eccentric exercise. the paper analyzes the relationship between the maximum contractile force and breaking load force of the gastrocnemius and the loss of the cytoskeleton protein and the change of the serum enzyme after the exercise induced the muscle injury. it also discusses the function of cytoskeleton protein in skeleton muscle fiber.

Key words: eccentric exercise; skeleton muscle; contractile character; material character; cytoskeleton

**1. Introduction:**

Many of the soluble growth factors, insoluble extracellular matrix (ECM) molecules and related membrane receptor signaling pathways that mediate tissue morphogenesis are now known. But we still cannot fully explain how these cues govern tissue formation, or how deregulation of these pathways lead to tissue breakdown and diseases. It is becoming increasingly clear that the Cytoskeleton, which generate mechanical tension that is transmitted across cell-ECM and cell-cell adhesion to other cell, play an

equally important role in development control.

**2. Research Purpose**

Skeletal muscle injury is characterized by an immediate loss of the ability of produce force. the cause of this force has been attributed to such factors as a defect in excitation contraction force (EC) coupling, disruption or loss of force generating structures such as actin and myosin and disruption or loss of force-transmitting structures, perhaps the disruption of force-bearing structures contribute to strength loss after injury come from Gastrocnemius study. Although the totality of injury is likely the result many factors for example dependent on its nerve supply, decrease of blood supply, Hormonal effect, Protein degradation. the purpose of this study was assess structural defects of the sarcolemma and ECM proteins after muscle injury and to relate these observations to contractile and material properties.

**3. Method and Material:**

Subjects: Male Wistar rats, weighing 300-360g. General test protocol: rats were performed an acute eccentric exercise. The acute eccentric exercise was -16° incline running on the treadmill at a speed of 26.8m/min, 5min×10groups with 1min rest. The rats were randomly distributed into one of six groups:

non-injury controls (NI, n=6) injury hour 0(H0, n=6), injury hour 4(H4, n=6), injury hour 12(H12, n=6), injury hour 24(H24, n=6), injury hour 48(H48, n=6), injury hour 72(H72, n=6), injury day 7(D7, n=6) ,The rat's feet was secured to a plate attached to a Miniature Materials Tester (model MiniMAT2000; made in USA). stimulation intensity to activate the gastrocnemius in our experiments to induce maximal contractile activation then breaking load force until the gastrocnemius was snapped.

The bone was dissected free through a small incision and clamped with a stimulator (Nokion made in Japan) that was used to stimulate the gastrocnemius with a supramaximal tetanic current (75-Hz 50V). We used this stimulation intensity to activate the gastrocnemius in our experiments to induce maximal contractile

activation then maximal force until gastrocnemius was snapped. Our protocol used commercial software (MiniMAT) to independently control the onset of contractile activation, proper velocity, and motion during plantar flexion

Transverse muscle sections (15µm) were cut at -20°C. Muscle fiber typing was diagnosed using metachromatic dye-ATPase methods. Dystrophin, Laminin-2, collagenIV, a-DG, DG content was determined by immunohistological stain methods. The expression of Dystrophin and Laminin-2 gene was detected by in situ hybridization with oligo nucleotide probe, digoxigenin labeled. Images acquired from stained sections were analyzed with Scion Image. CK, LDH in serum, SOD, MDA in muscular tissue were measured respectively.

#### 4. Result:

Average mass of rats in different group

groups	NI	H0	H4	H24	H48	D7
Body weight	333.50±15.71	319.00±16.60	325.17±15.52	333.00±21.17	342.67±11.52	356.17±31.54

The efflux of Serum enzymes CK, LDH

Groups	mSOD (U/mgprot)	mMDA (nmol/mgprot)
NI	6.28±2.12	0.32±0.04
H0	9.08±1.64	0.74±0.53
H4	9.48±2.65	0.66±0.50
H12	10.28±2.34	0.93±0.60
H24	8.21±1.14	0.34±0.55
H48	9.56±1.95	0.37±0.44
H72	7.34±2.28	0.26±0.16

Maximal tetanic tension

Groups	Section(C)	MTT(ssl)	(ssl/C)
NI	1.59±0.41	10.54±1.39	6.92±1.68
H0	1.40±0.19	5.88±0.95	4.19±0.39
H4	1.42±0.34	10.04±5.54	6.99±3.44
H24	1.54±0.19	7.43±0.78	4.88±0.72
H48	1.61±0.18	6.67±3.06	4.22±1.99
D7	1.52±0.20	13.61±4.63	8.83±2.18

To evaluate the loss of contractile function after exercise-induced injury, we measured maximal tetanic tension (MTT) within 15 min after the injury (H0) and at H4, H24, H48, Day7. In non-injured controls, Po was 6.92±1.68, and within 15 min after the eccentric contraction, maximal tetanic tension decreased to 4.19±0.39\*. Tetanic tension recovery to 6.99±3.44 # at

H4, then tetanic tension continued to decrease 4.22±1.99\* at H48, This further decline in muscle force has been noted in other studies and has been termed a “secondary injury”. likely due to inflammation. Tetanic tension reach 8.83±2.18 # at day 7.

Breaking load force(BLF)

Groups	Section(C)	BLF(ldl)	(ldl/C)
NI	1.59±0.41	50.63±4.95	32.99±5.78
H0	1.40±0.19	49.50±9.49	35.16±4.66
H4	1.42±0.34	53.61±10.58	38.45±6.50
H24	1.54±0.19	40.95±3.40	27.06±5.11
H48	1.61±0.18	48.51±6.62	30.48±5.42
D7	1.52±0.20	59.14±5.65	39.20±3.18

Myosin content and Semiquantitative analysis

groups	NI	H0	H4	H12	H24	H48	H72	D7
	2888±1071	1827±778	939±339	754±222	1564±187	515±294	1332±146	4176±1633

Tissue section from Gastrocnemius were labeled for proteins and Semiquantitative analysis

groups	Dystrophin	Laminin-2	CollagenIV	a-DG	B-DG
NI	1640.18±539.34	2694.50±506.81	731.70±111.95	1941.38±682.74	2034.68±333.89
H0	1446.36.80±287.51	2804.80±561.28	763.36±116.31	1505.50±347.72	1820.60±344.15
H4	1182.12±432.48	2568.60±903.49	732.07±99.82	1294.00±351.18	1668.82±666.51
H24	1237.26±332.84	4032.13±430.93	725.93±56.86	1303.67±267.78	1879.98±365.56
H48	1214.93±472.76	2510.00±936.04	610.78±116.22	1284.50±77.29	1928.26±129.13
H72	21031.08±467.42	2790.47±402.56	650.50±55.75	1342.75±129.91	1775.40±261.66

The expression of laminin-2 gene was detected by in situ hybridization with oligo nucleotide probe, digoxigenin labeled

Groups	OD (Dystrophin)	Gene expression	OD (laminin-2)	Gene expression
NI	1617.12±1464.55	100%	1654.11±751.62*	100%
H0	448.96±578.67	28%	862.75±50.12	52%
H4	958.06±1068.20	59%	2352.75±489.10	142%
H24	1349.16±1383.18	83%	2302.36±325.56	139%
H48	2781.04±430.94	172%	2538.56±213.18	153%
H72	1523.48±1006.02	94%	2193.08±659.33	133%

## 5. Discussion & Conclusion

As we know, all tissue has a characteristic ability to tolerate deformation and stress, and injuries occur when this tolerance level is exceeded. Acute injuries occur when tissue loading is sufficient to cause sudden irreversible deformation of the tissue or injuries occur as a result of repeated overloading, each incidence of which, alone, is not enough cause irreversible deformation, but which when accumulated over time exceeds the tissue injury threshold.

### 5.1 Laminin-2 Plays an Important Role in BM Assembly

Extracellular matrix (ECM) is the extracellular part of animal tissue that usually provides structural support to the animal cells in addition to performing various other important functions. Skeletal muscle basal lamina is linked to the sarcolemma through transmembrane receptors, including laminin-2 and dystroglycan. When sarcolemma injury is happen, molecular mechanisms could be regulation of Laminin-2 Adhesion and Signaling - Inside-Out Regulation, extracellular agonists (growth factors, cytokines, etc.) stimulate intracellular signals that “activate” laminin-2 extracellular adhesion. Most laminin-2 on the cell surface are “inactive”. Some engineered antibodies distinguish “active” from “inactive”. Our results suggest that laminin-2 might play an important role in that contribute to the synthesis of other proteins due to the animal tissue was

in weakest but laminin-2 protein expression strongly in the meanwhile.

### 5.2 Dystrophin and a-dystroglycan play an important role in force-transduction pathway

The present study examined the extent of membrane and cytoskeletal damage in skeletal muscle fibers after eccentric contraction. The organization of dystrophin and a-dystroglycan was selectively affected, whereas that of its associated molecules, the organization of  $\beta$ -dystroglycan was affected to a much lesser extent compared with dystrophin and a-dystroglycan. On the other hand, the loss of dystrophin organization may not be solely due to a direct mechanical disruption of elements supposedly responsible for force transduction. There have been reports that dystrophin is vulnerable to calcium-activated proteases .Because we show in our model of muscle injury that the integrity of the membrane is seriously compromised, it is reasonable to assume that extracellular calcium enters the muscle fiber. Disruption of both the sarcolemma and internal cytoskeleton has previously been implicated in loss of force production after muscle injury .Force is generated within sarcomeres, and it is generally accepted that this force is transmitted longitudinally along the myofibril. However, there is evidence that force is also transmitted radially through intermediate filaments (e.g.,desmin) of the internal cytoskeleton and outward toward the sarcolemma. We know that dystroglycan contribute to force-production of muscles,

the disruption of dystroglycan causes detachment of the basal lamina from the sarcolemma and renders muscle prone to contraction-induced injury. In the present study, we have shown a previously uncharacterized mechanism that the skeletal muscle cells use to strengthen the sarcolemma integrity, anchoring the sarcolemma to the basal lamina via laminin G domain-binding motif on  $\alpha$ -dystroglycan. By imposing an eccentric contraction, the high forces generated may strain components of the putative force-transduction pathway. Our results suggest that dystrophin and  $\alpha$ -dystroglycan play an important role in that pathway

Therefore, DG-dependent tight physical attachment of the basal lamina to the sarcolemma is important for

transmission of the basal lamina's structural strength to the sarcolemma to provide resistance to mechanical stress. Collectively, our data suggest that the basal lamina is tightly associated with the sarcolemma through DG binding to the LG domains of the basal lamina proteins of skeletal muscle. Lengthening contractions cause an increase in membrane tension on the sarcolemma, which can lead to small tears in the membrane. The membrane repair mechanism subsequently reseals these membrane tears and thus restores the membrane integrity of myofibers. Our findings support the idea that reinforcement of the basal lamina-sarcolemma attachment is a basic cellular mechanism that allows cell survival in tissues subjected to mechanical stress.