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A HIERARCHICHAL MODEL OF A QUIESCENT AND ACTIVATED SKELETAL MUSCLE FIBER DURING STRETCH

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ABSTRACT

Although techniques exist to isolate and study regions of a skeletal muscle fiber in which injury has taken place, a definitive method for isolating the mechanism of injury at the level of a single sarcomere has not been accomplished. The aim of this study was two-fold: (1) to develop a structural model of a single fiber in order to determine the evolution of stress and strain during the course of an injury inducing protocol, (2) to test the hypothesis that the mechanism of injury is first heterogeneity in the active force generation of adjacent sarcomeres resulting in damage to passive structures. The homogenization method provided the stress/strain response for both a quiescent and activated single fiber during stretch. The peak strains and total strain energy (TSE) at the myofibril level were located in the Z-disc. For a constant strain rate of 2 fiber lengths per second from 0% to 10% strain in the quiescent and activated models, the TSE for the interfibrillar proteins was found to be 8.27 and 8.31 nN/μm^2, respectively. These data demonstrate that in an uninjured fiber, the interfibrillar proteins play a negligible role in transferring forces and experience low strains. We conclude that the primary mechanism of injury is heterogeneity in the active force generation of adjacent sarcomeres resulting in secondary damage to the passive structures.

NOMENCLATURE

Γ:	boundary of solid Ω
Ω:	solid body
σ_a:	active stress applied to solid Ω
χ:	characteristic deformation tensor for the RVE due to the global deformation
ε:	superscript denotes dependence on the microstructure
ϕ:	displacement in the RVE due to the active stress
ρ:	material mass density
$\boldsymbol{A}$:	vector {1 1 1 0 0 0}
$\boldsymbol{D}$:	material constitutive matrix
H:	superscript denoting homogenized quantities with respect to the microstructure
$\boldsymbol{b}$:	body force vector per unit mass
f:	rate function for cross-bridge attachment
g:	rate function for cross-bridge detachment
n:	population distribution function for attached cross-bridges in a half sarcomere
p:	Lagrange multiplier
r:	sufficiently small positive number
$\boldsymbol{t}$:	traction vector on boundary Γ^t
$\boldsymbol{u}$:	displacement vector
v_r:	relative velocity of shortening in a half-sarcomere
L_f/sec:	Fiber lengths per second determined relative to length at which peak force is generated.
nN/μm^2:	nano-Newtons per square micrometer
RVE:	Representative volume element representing the material microstructure.
TSE:	Total strain Energy

INTRODUCTION

Pliometric (lengthening) contractions are more likely to cause injury than either isometric (fixed length) or miometric (shortening) contractions, but the behavior of muscle fibers during injury and the exact mechanism by which injury occurs have not been demonstrated conclusively. For specific protocols, several mechanical factors correlate well with measures of injury. These strong relationships do not persist when the protocol for imposing the injury is changed.[1,2] Although techniques exist to study the mechanism of injury, resolving injury at the level of a single sarcomere has not been accomplished.[3]

The purpose of this study was to develop a computational model of a single fiber from rat soleus muscle that reflects the changes in the mechanical environment of individual sarcomeres and their response to imposed loads. We hypothesize that the mechanism of injury is heterogeneity in the active force generation of adjacent sarcomeres resulting in increased loading and damage to passive structures. The hypothesis will be supported if during stretch the total strain energy is greater in the intrafibrillar regions along a myofibril than in the interfibrillar proteins which connect parallel myofibrils.

ANALYSIS METHOD

Solid Model

The homogenization method with a Newton-Raphson iterative solver was used to derive the formulation for an incompressible, linear elastic, two scale model with an active stress.[4,5,6] The augmented Lagrangian for an incompressible material with a residual stress in its microstructure is defined by the following equation:

$$\begin{aligned} L_r\left(\mathbf{u}^\varepsilon, p^\varepsilon\right) = & \frac{1}{2}\int_{\Omega^\varepsilon}\left(\partial\mathbf{u}^\varepsilon\right)^T \mathbf{D}^\varepsilon\,\partial\mathbf{u}^\varepsilon\, d\Omega \\ & - \int_{\Omega^\varepsilon}\left(\partial\mathbf{u}^\varepsilon\right)^T \sigma_a^\varepsilon\, d\Omega - \int_{\Omega^\varepsilon}\mathbf{u}^{\varepsilon T}\rho^\varepsilon\,\mathbf{b}^\varepsilon \\ & - \int_{\Gamma^\varepsilon}\mathbf{u}^{\varepsilon T}\,\mathbf{t}\,d\Gamma - \int_{\Omega^\varepsilon}\left(\partial\mathbf{u}^\varepsilon\right)^T \mathbf{A}^T\, p^\varepsilon\, d \\ & - \frac{1}{2}\int_{\Omega^\varepsilon} r^\varepsilon\left(p^\varepsilon\right)^2 d\Omega \end{aligned}$$

The solution to the minimization problem for the asymptotic expansion of the augmented Lagrangian at the microlevel leads to the following homogenized quantities at the global level:

$$\mathbf{D}^H = \frac{1}{|Y|}\int_Y \mathbf{D}\left(\mathbf{I} - \partial_y\chi\right) d$$

$$\sigma_a^H = \frac{1}{|Y|}\int_Y \left\{\left(\mathbf{D} + \mathbf{A}^T r^{-1}\mathbf{A}\right)\partial_y\phi - \sigma_a\right\} d$$

$$p^H = \frac{1}{|Y|}\int_Y r^{-1}\mathbf{A}^T\mathbf{A}\left(\mathbf{I} - \partial_y\chi\right) d$$

These homogenized quantities in turn are used to solve the global level problem. The representative volume element (RVE) shown in Figure 1 is used to model the repeating microstructure of the global muscle fiber solid model.

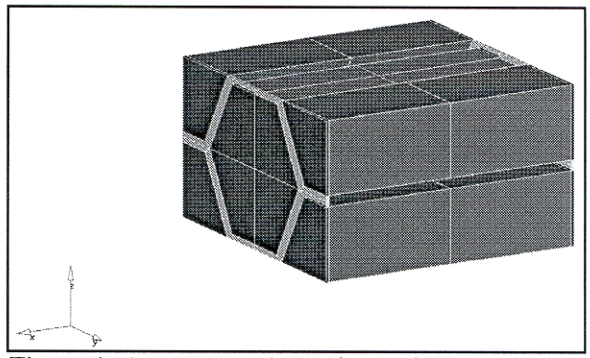

Figure 1: A representative volume element model of the microstructure showing the Z-disc (black), interfibrillar proteins (light gray), and the contractile zone (dark gray).

Active Properties

The active stress was determined using the Huxley contraction model and incorporating Zahalak's modification to account for lengthening of a sarcomere.[7,8] A constant strain rate at the global level was assumed to result in a constant strain rate at the microlevel. Based on this assumption, the active stress

$$\sigma_a(t) = C_1 \int_{-\infty}^{\infty} xn(x,t\ d$$

was calculated by solving the Huxley equation

$$\frac{\partial \mathrm{n}}{\partial t} - v_r\frac{\partial \mathrm{n}}{\partial x} = f(x) - \left[f(x) + g(x)\right.$$

for each half-sarcomere in the RVE model while using the forward Euler technique to determine the velocity. The constant C_1 was scaled to match stress data reported for single myofibrils activated isometrically.[9] The rate constants for f(x) and g(x) were taken from experimental work performed by Wahr and Metzger on rat soleus muscle.[10]

Passive Properties

The passive elastic properties for a sarcomere were approximated from stress versus stretch velocity curves reported in the literature.[11,12] These data were then scaled from the single fiber level to the level of a single titin molecule via the ratio of cross-sectional areas. Based on the properties of titin, a model was constructed to determine the passive elastic moduli for the contractile zone defined as the region from Z-line to M-line. The elastic moduli for the interfibrillar proteins and the Z-disc were approximated as $2.0\text{x}10^3$ and $2.5\text{x}10^6$ nN/μm^2 respectively.

A linear analysis was performed for a solid model of a single fiber subject to a constant velocity stretch of two fiber lengths per second. The initial sarcomere length was defined as 2.05μm. The stretches of a quiescent fiber, a maximally activated fiber, and a maximally

activated fiber containing sarcomeres with 75% of maximum contractility were considered

RESULTS

Material Properties

The elastic modulus for the region from Z-line to M-line was dependent both on fiber length and stretch velocity. For a stretch velocity of 2 L_f/sec, the elastic modulus of the region from Z-line to M-line was approximately 3.68×10^2 nN/μm^2. The initial active force generated in a single myofibril was approximately 63 nN/μm^2. The peak active force during the 2 L_f/sec was approximately 285 nN/μm^2. The resulting force versus fiber length curves are shown in Figure 2.

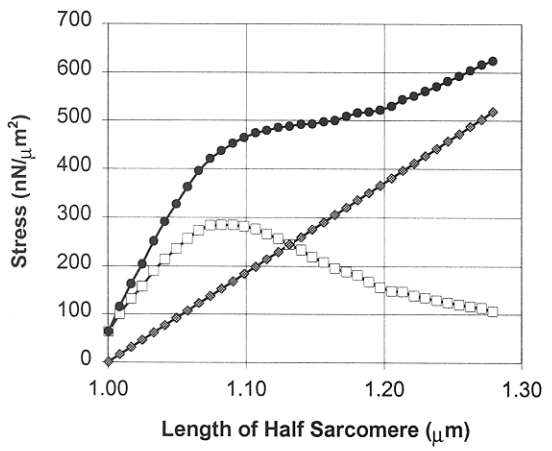

Figure 2: Numerical approximation of passive force (♦), active force (□), and total force (●) for the stretch of a myofibril at a rate of 2 Lo/sec.

Solid Model

Stress and strain fields, including the TSE, were calculated for the RVE of each myofibrillar solid element. The stresses in the maximally activated fiber model were greater than the stresses found in the quiescent fiber model. A constant global strain rate of 2 L_f/sec from 0% to 10% strain results in the TSE data shown in Table 1.

Table 1: Total Strain Energy (nN/μm^2)

Material	Passive	Active	Injury
Interfibrillar	8.27	8.31	9.32
Z-disc	894	901	1080
Contractile Zone	2.48	2.48	19.4

The peak strains within the RVE were located in the region corresponding to the M-line of the sarcomere. For the constant velocity loading from 0% to 10% strain at a strain rate of 2 L_f/sec in both the activated and quiescent models, the maximum TSE was found in the Z-disc (Fig 3,4,5). The peak active stress of 142 nN/μm^2 was located in the contractile zone. The peak passive stress of 36,400 nN/μm^2 was located at the Z-disc.

DISCUSSION

Solid Model

The results for the total strain energy demonstrate that in an uninjured fiber the interfibrillar proteins connecting parallel myofibrils play a negligible role in transferring forces and experience low strains. In order for the interfibrillar proteins to experience loading, a difference in the length of the sarcomeres connected by those proteins would have to exist. The injury model that simulates a 25% force reduction in a region of the fiber (Fig 3) leads to a rise in the strain and TSE for the contractile zone as well as the interfibrillar proteins in that region. These observations support our hypothesis that the primary mechanism of injury heterogeneity in the active force generation of adjacent sarcomeres leads to increased loading and damage of passive structures.

The results of the solid model at the level of the myofibril show that the peak active stress is found at the M-line. The peak stress and TSE for the model are located in the region corresponding to the Z-line. These findings are expected since horizontally opposing forces intersect at these histological landmarks. The magnitude of the TSE values for the Z-disc are the result of the large elastic modulus assumed for this material relative to the interfibrillar proteins and the contractile zone. The Z-disc is a lattice structure composed primarily of α-actinin which is some forty times stiffer than actin. To more accurately represent the material properties of the Z-disc, a more detailed model of the Z-disc structure is required.

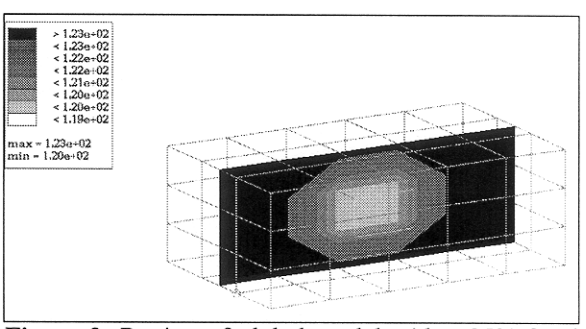

Figure 3: Region of global model with a 25% focal reduction in active force showing "injury bubble".

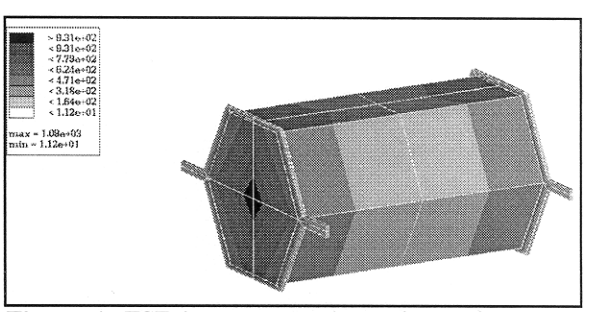

Figure 4: TSE in representative volume element of an injured myofibril.

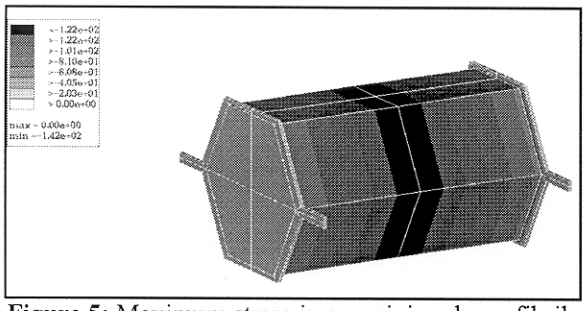

Figure 5: Maximum stress in an uninjured myofibril

Force-Length Curve and Stability

The experimental data reported for stress as a function of velocity shows a significant difference between peak stress values and stress measured after relaxation has taken place.[11] These data demonstrate that during the course of a stretch the passive elastic properties are velocity dependent. In the range of sarcomere lengths calculated in this model, the average elastic modulus for a stretch rate of 2 L_f/sec results in a system that is stable on the descending limb of the length-tension curve (Fig 2).

Experimental data show a very small toe-in region leading to a sharp rise in the passive length-tension curve.[9,11] A low initial toe-in region would likely result in a lower total stress and a lower TSE in each of the materials in this model. A reduction in the passive stress in this region would also cause instability for large strains (>10%). Bartoo *et al* reported instability in their single myofibril experiments at high strains where some sarcomeres would shorten at the expense of other sarcomeres being stretched beyond overlap of thick and thin filaments. The computational model also displayed these characteristics where sarcomeres became numerically unstable when heterogeneity was added to the model for activation and stretch of a single myofibril. The addition of parallel myofibrils to the unstable myofibril lead to a stable system with high strain and TSE values in the region of injury. Further work is necessary to fully quantify the dimensions of the ellipsoid or "injury bubble" associated with different types of injury models.

CONCLUSION

The homogenization theory was able to approximate the stresses and strains at the level of the sarcomere. The results supported the hypothesis that the primary mode of injury is damage to the contractile apparatus which leads to increased loading and possible injury of passive structures. Future theoretical work should include: i) a more detailed analysis of the structure of the Z-disc, ii) large deformation theory to account for high strains (>10%) that occur in single fibers during experiments which induce injury, iii) the non-linear, viscoelastic response of the passive proteins within the myofibril.

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REFERENCES

1) Hunter and Faulkner (1997) *J. Appl. Physiol.*, **82**, pp. 278-283.
2) Lieber and Fridén (1993), *J. Appl. Physiol.*, **74**, pp. 520-526.
3) Macpherson *et al* (1997), *J. Physiol.*, **500(pt2)**, pp. 523-533.
4) Hollister and Kikuchi, (1994), *Biotech. Bioeng*, **43**, pp. 486-586.
5) Heguri (1995), Ph.D. Thesis, Univ of Michigan.
6) Kikuchi, "Foundation of the Homogenization Methods for Mechanics of Composites".
7) Huxley (1957), *Progress in Biophysics*, **7:255**.
8) Zahalak and Ma (1990), *J. Biomechanical Eng.*, **112**, pp 52-62.
9) Bartoo *et al* (1993), *J. Muscle Res. Cell Motil.*, **14**, pp. 498-510.
10) Wahr and Metzger (1997), *Biophys. J.*, **72**, pp. 822-834.
11) Mutungi and Ranatunga (1996), *J. Physiol.*, **496.3**, pp. 827-836
12) Higuchi (1993), *Biophysics J.*, **65**, pp. 1906-1915