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An *In Vivo* Animal Model for the Investigation of Acute and Chronic Skeletal Muscle Injury

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Abstract. An *in vivo* animal model to study skeletal muscle injury is described. A computer-controlled custom-designed rat dynamometer is used to control biomechanical inputs such as range of motion, velocity, acceleration, and number of repetitions to study skeletal muscle injury in rats. Anesthetized rats are placed supine in the dynamometer and the left foot placed in a load cell with the ankle axis aligned with the axis of rotation of the motor. Platinum electrodes are placed subcutaneously to branch either the peroneal nerve (to activate the dorsi flexor muscles of the hind limb) or the tibial nerve (to activate the plantar flexor muscles of the hind limb). The free ends of the wire electrodes are connected to a computer-controlled nerve stimulator. The dynamometer can be programmed to produce controlled angular movement about the ankle axis to generate isometric, concentric, and reciprocal concentric/eccentric muscle actions of either the plantar flexor or dorsi flexor muscles. This model has distinct advantages as compared to invasive *in vitro* or *in situ* preparations of isolated muscles or muscle fibers. Muscle response and injury can be studied in a more physiologically representative fashion with the neural and vascular supplies and muscle-tendon complexes intact. The non-invasive features of this model are well suited for the study of chronic muscle injury. When the biomechanical data from the dynamometer are combined with histological and biochemical analyses of muscle tissue, this model can provide comprehensive data for studying acute and chronic skeletal muscle pathomechanics.

1. Introduction

The significance of muscle injuries or strains is readily apparent for those treating sports or work-related injuries. The economic impact of work related injuries is detrimental to industry as well as the employee involved [1]. Attention is now directed toward the ergonomic design of the workplace in an attempt to alleviate or reduce job-related injury. There has been a rapid growth of scientific understanding of injury and recovery of bone, ligament and cartilage, yet little is known of the factors that predispose muscle to injury or the mechanism that produces symptoms. There is an obvious need to better quantify dynamic muscle performance and the mechanism of injury responsible for reduced muscle performance. If the dynamic parameters that will produce injury such as velocity and acceleration, coupled with force, number of repetitions, and range of motion are quantified, the workplace or sports medicine personnel could design work practices under the injury threshold. Biomechanical studies indicate muscle injury occurs at forces greater than the maximal isometric force, and muscle stretch is necessary to produce injury [2, 3, 4]. If muscle is injured by excessive force development in the muscle-tendon unit, higher forces are possible with eccentric (lengthening) contractions [2, 5]. New investigative methods must be developed to elucidate the functional and physiological events associated with muscle injury.

The use of a dynamometer has aided this field of study since strain, strain rate, number of

repetitions, and force can be accurately quantified to determine the dynamic pa responsible for injury. The Kin Com and other dynamometers have recently been human studies to yield quantitative information of torque, power, and velocity in isoki isotonic modes [6]. Human studies have indicated that despite the low metaboli eccentric contractions, they cause profound changes in muscle structure and Recovery from eccentric contraction- induced injury is a prolonged process [7].

Most attempts to study the mechanisms of muscle injury have used biolo mechanical markers of muscle injury measured before and/or after injury occurred i hypothesize how injury actually occurs. Studying the mechanisms of muscle injury a is difficult because of the dynamic contractile properties of skeletal muscle. I advancements in dynamometry have allowed for precise control and quantifi mechanical parameters associated with muscle injury as it occurs in real-time. Quan of the mechanical parameters associated with muscle injury like strain, strain rate, should elucidate the mechanisms of muscle injury. A non-invasive method v advantageous to characterize the dynamic response of skeletal muscle and quantify the parameters necessary to produce injury.

The scientific literature indicates that animal models are appropriate for the inv of skeletal muscle injury [8]. The use of anesthetized rats allows for controlled stim the muscle group of interest and rigorous histological and biochemical analysis of th tissue. Two isokinetic rodent dynamometers have been designed and fabricat laboratory to facilitate *in vivo* muscle testing of rodents. The rats will be selected fro outbred stock and age-matched. The use of rats in this study will provide the c environment necessary to conduct a parametric investigation of the factors that affect chronic strain overload injury. The micro-architecture of rodent and human skeletal quite similar. The disadvantage of human *in vivo* testing is the inability to contr activation, inability to remove and dissect the involved muscle-tendon groups for s analysis, and determination of the histopathological process and the site(s) of previous studies, the human and rodent response to acute injury resulted in similar pe decrements, and biochemical expression [6].

This research will focus on the etiology of acute and chronic strain overload i repetitive strain injury). To date, systematic, quantitative studies of acute injury l conducted on both humans and animals (rats, mice, and rabbits) using constan movements [7, 9, 2]. However, no study to date has investigated the pathophysiolog injury due to oscillatory loading or the resultant cytokine expression. Also, changes performance, muscle tissue structure, and biochemical expression of proteins ar enzymes in the serum and urine have not been investigated during chronic contractio injury. By investigating these phenomena, this research program should eluc pathomechanics of acute and chronic contraction-induced muscle injury.

2. *In Vivo* Rodent Dynamometry

In vivo dynamometry is well suited for the study of muscle mechanics in rodent dynamometry is designed to test the performance of a muscle group in a relatively no fashion where the neural and vascular supply and muscle tendon complexes are int muscle performance is assessed by measuring torque about the target joint axis developed by a specific muscle group about the joint axis. There are distinct advan *vivo* dynamometry over *in situ* and *in vitro* models of muscle performance a Classically, *in vitro* models (where the target muscle is excised and placed in a phy bath) were used to test contractile function of muscle [10] and then later used to i muscle injury [11]. While this model provides accurate information about the contractile function of isolated muscle during a single exposure, the muscle is physiologically compromised since the neural and vascular supplies have been removed. In addition, the effect of adjacent agonist muscles, the effect of tendon mechanics, and the biomechanical performance about the joint axis cannot be investigated. This model is also not suited to study chronic skeletal muscle injury. *In situ* models have been widely used to study skeletal muscle function and injury. *In situ* models (where the distal tendon has been ligated and contractile function is assessed at the tendon) were developed such that a target muscle could be tested with the neural and vascular supplies intact. These studies have provided in-depth information about acute injury mechanisms and the impact on muscle function [9, 12]. However, this procedure is invasive and does not assess the biomechanical performance about the joint axis or incorporate the effect of agonist muscle action. This model has only been used for acute studies due to the invasiveness of the preparation. In contrast, *in vivo* models are well suited for the study of acute and chronic muscle performance and injury due to the intrinsic non-invasive nature of the procedure. This model is physiologically representative since it allows for the study of muscle function via the biomechanical performance about the joint axis, and incorporates agonist muscle function and tendon mechanics.

To date, rodent dynamometers have been designed to test either the dorsi flexor or plantar flexor muscles in the lower limb [13, 14]. Muscle performance can be assessed under both static (isometric) and dynamic (concentric and eccentric) conditions in anesthetized rodents. Also, the effect of dynamic parameters on skeletal muscle injury (velocity, force, range of motion, number of repetitions) can be investigated.

3. Apparatus

Two rodent dynamometers designed and fabricated at NIOSH were specifically designed to quantify the static and dynamic muscle response of anesthetized rats *in vivo* (Figure 1). The dynamometers were designed to operate in isometric (static muscle length), isokinetic (muscle changing length at constant velocity), and controlled non-isokinetic (muscle changing length during constant or changing acceleration) modes where range of motion, angular motion of the lower limb, and electrical stimulation of either the plantar flexor or dorsi flexor muscles are controlled [14]. The dynamometers have the capability of providing isometric, shortening, and lengthening actions of the plantar flexor and dorsi flexor muscle groups. The platform of the dynamometer is an aluminum table that holds all the testing components, such as the Aerotech DC servomotor and tachometer, a micrometer-driven animal positioning platform (positioning within 0.025 mm with a positional repeatability of 0.002 mm), load cell fixture, and potentiometer. The dynamometers were designed for rats to be placed supine on a heated positioning platform (to maintain each animal's body temperature) with the foot being placed onto a load cell fixture. The knee is secured in a 1.57 rad (90 degrees , tibial-femoral angle) orientation by a custom designed knee holder. A DC servomotor will provide either isometric, or controlled dynamic movements within the preprogrammed range of motion while the muscle group of interest is either stimulated or non-stimulated. The load cell provides muscle output forces during the isometric and controlled dynamic movements in real time. Computer programs were developed using Labview software to interface with the operator, communicate with the motion controller for control of the DC servomotor, collect data, activate the electrical stimulator, and display procedural data on the screen in real time.

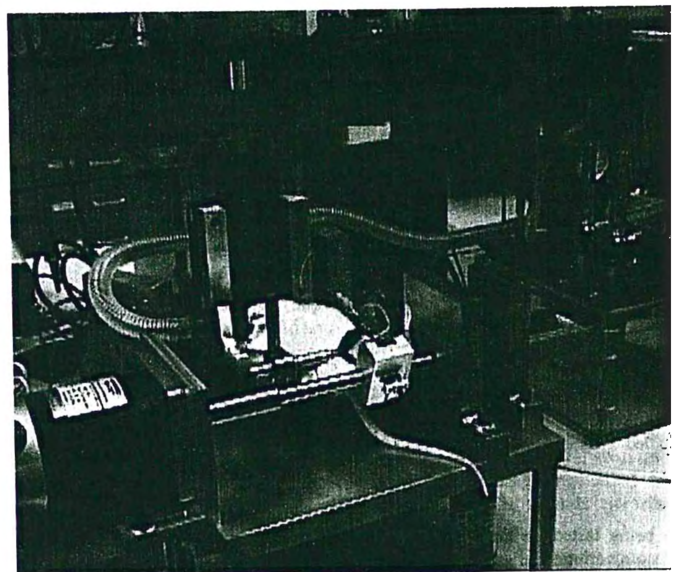

Figure 1. In Vivo Rodent Dynamometer complete with anesthesia system, DC servomotor, p micrometer-driven animal positioning table, load cell fixture and knee holder.

To measure the muscle forces at the plantar surface of the foot, an aluminum fabricated to hold a strain gage load cell (Sensotec model 13, 25 lb capacity non-linearity and hysteresis of 0.25%-0.5% full scale) and the animal's foot. Th load cell is designed for measurement of dynamic signals with a natural frequenc and response from zero load to full scale (25 lbs rated) in approximately 24 milli

To translate the force exerted by the foot into a purely vertical movement re load cell transducer, a 40 gram rectangular aluminum plate is mounted on four lin (THK CO, LTD, Tokyo, Japan). Measurement of the dorsi flexor forces at the dc of the foot is via a different load cell fixture complete with a Sensotec Model 13 lc two linear bearings (THK CO, LTD, Tokyo, Japan). Each load cell was connecte amplifier/signal conditioner (MVD 2555, HBM Inc., Marlboro, MA) and 16 bi (National Instruments Inc, Austin, TX) in the host computer for sampling at 5 potentiometer, used for angular measurement of the load cell fixture, has a range The potentiometer is sampled at 500 Hz.

The DC tachometer, which is used to record angular velocity of the load ce connected directly to the DC servomotor. It produces a linear voltage of 12 revolutions per minute, with an output ripple of 1.5% RMS, a ripple freq cycles/rev, and 0.2% linearity. The tachometer is also sampled at 500 Hz.

Electrical stimulation of the plantar flexor and dorsi flexor muscle groups is a Grass SD-9 stimulator (Grass Medical Instruments, Quincy MA) and subcutane needle electrodes. Typical stimulation voltages are less than 6 volts with a 0.2 pulse duration and 120 Hz stimulation frequency. Stimulation of the muscl controlled by the computer via a single digital line and can be turned on and off as a function of either time or position of the load cell fixture.

The motion control of the shaft, and consequently the load cell fixture and the rat's foot is accomplished by an DC servo with a 2000 line quadrature output encoder and Unidex 100 motor controller unit (Aerotech Inc., Pittsburgh, PA). Isometric, isovelocity, and complex motions involving acceleration and jerk are controlled by an Aerotech Unidex 100 servo motion controller. The Unidex 100 quadruples the quadrature output from the encoder on the servo for a feedback resolution of 8000 counts/revolution to monitor shaft position, and contain internal PID loops for adjusting servo performance characteristics. After the host computer communicates via an RS-232 port the desired motion of the servo, the operation of the motor controller is independent of the host computer. An entire series of tests can be downloaded to the motor controller including servo motion, delays, and repetition number.

The DC servo model 1410 DC produces a peak torque of at least 10 times the maximum torque that a rat can produce via the load cell fixture. A rat of approximately 400 grams should be able to exert a maximum force of 30 N [14] that produces a corresponding torque of approximately 0.75 N-m. These values were verified from animal testing on the rat dynamometer. As the torque generated by the animal is negligible compared to the torque capability of the servomotor, performance testing and optimization of the motor control loop can be conducted without an animal.

4. Procedure

Prior to being tested on the dynamometer, each animal (male Sprague Dawley) is anesthetized via a commercially available rodent anesthetic system (Surgivet Anesco) that provides the proper mixture of oxygen and isoflurane gas. Animals are first placed in an enclosed "induction" tank that was filled with a mixture of isoflurane gas and oxygen. Each animal is then placed supine on the heated x-y positioning table of the rodent dynamometer [14] while a mask is placed over the animal's nose and mouth (Figure 1). The knee is secured in flexion (at 1.57 rad, 90 degrees) using the knee holder. The left foot is secured in the load cell fixture with the ankle axis (assumed to be between the medial and lateral malleoli) aligned with the axis of rotation of the load cell fixture. Each animal is monitored during the procedure for proper anesthetic depth. The platinum stimulating electrodes are then placed subcutaneously so as to span either the peroneal nerve (to stimulate the dorsi flexors) or the tibial nerve (to stimulate the plantar flexors).

4.1 Isometric Testing

Isometric testing of either the plantar flexor or dorsi flexor muscles is necessary to characterize the static performance of the muscle group. To achieve this, the load cell can be secured at any ankle angle (0.52 rad - 2.97 rad, 30 – 170 degrees). The muscle group of interest can be maximally activated via the electrical stimulator for a duration of 1.0 second. The electrical stimulator is then deactivated which allows for muscle relaxation. The duration of the isometric contraction can be programmed to accommodate a variety of testing objectives. Muscle fatigue during long term isometric contractions can be studied with this device. The dynamic muscle forces also are recorded such that contraction time, relaxation time, fatigue, and maximum isometric forces can be analyzed. Thus, the dynamometer is capable of multi-positional isometric testing where maximal isometric forces and the dynamics of muscle contraction, fatigue, and relaxation can be analyzed. In addition, isometric tests of 1.0 second duration are used to determine the change in muscle performance at selected time points after exposure to either long term isometric contractions, concentric contractions, or oscillatory contractions.

4.2 Concentric Testing

Concentric testing of the plantar flexor or dorsi flexor muscles is used to charac
muscle performance during dynamic movement conditions. Concentric testing
dynamometer is accomplished by maximally activating the muscle group for 150 mil
to allow for maximum force generation. The load cell is then moved via the servo
pre-programmed angular velocity (0.52 rad/s - 17.5 rad/s, 30 deg/s – 1000 deg/s) th
range of motion selected (0.17 rad – 2.44 rad, 10 deg – 140 deg) to allow f
shortening. Motion is then terminated and the electrical stimulator is deacti
milliseconds later which allows for muscle relaxation. The dynamometer ca
programmed to provide multiple concentric muscle actions at varying time in
determine the effect of sequential concentric muscle actions and work-rest cycles
response.

4.3 Eccentric Testing

Eccentric testing of the plantar flexor or dorsi flexor muscles is beneficial in e
the response of muscle actions that have been shown to be injurious in previous a
human studies. Eccentric testing is accomplished via maximally activating the mu
for 150 milliseconds. The load cell is moved via the servomotor at a pre-programm
velocity (0.52 rad/s - 17.5 rad/s, 30 deg/s – 1000 deg/s) through the range of motio
(0.17 rad – 2.44 rad, 10 deg – 140 deg) to produce active muscle lengthening. Mo
terminated and the electrical stimulator is deactivated 150 milliseconds later which
muscle relaxation.

The dynamometer can be programmed in a similar manner as for the concentri
administer multiple eccentric muscle actions at varying time intervals to determine
sequential high force eccentric muscle actions and work-rest cycles on muscle inj

4.4 Oscillatory Testing

Since most occupational tasks require both eccentric and concentric muscle
dynamometer was designed to also accommodate oscillatory testing
eccentric/concentric muscle actions). Oscillatory testing is accomplished on the dy
by maximally activating the muscle group 150 milliseconds before movement is in
load cell is moved via the servomotor at a preprogrammed angular velocity (0.52
rad/s, 30 deg/s – 1000 deg/s) and range of motion (0.17 rad – 2.44 rad, 30 deg – 1
reciprocal fashion (clockwise and counterclockwise motions) while the muscle is a
termination of the motion, the electrical stimulator is deactivated which result
relaxation. During normal operations, the dynamometer is programmed to
successive repetitions. The dynamometer is normally programmed to deliver succ
10 repetitions at varying time intervals. The dynamic muscle forces during th
movements can be decomposed to analyze the isometric and eccentric force com
their respective decay during the set. Phase shifts in the muscle response
quantified. The user can program the dynamometer to deliver multiple sets c
contractions. The oscillatory procedure has been used reliably in our laborator
muscle injury of the dorsi flexor muscles. Evidence of fiber necrosis and inflamm
the endomysial and perimysial spaces, indicative of muscle injury, has resulted a
to oscillatory contractions (Figure 2).

4.5 Muscle dissection and preparation

Use of a rat model employing the tibialis anterior muscle (TA) results in a tissue sample of approximately 800 mg. supplying adequate sample size to investigate outcomes with a variety of techniques such as histology, enzyme assays and gene expression.

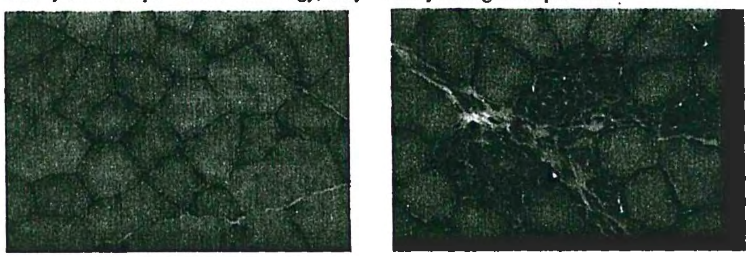

Figure 2. Uninjured TA muscle 72 hours after repeated isometric contractions (left). Injured TA muscle 72 hours after repeated oscillatory contractions (right). H&E stain with 40x objective.

5. Conclusions

The application of *in vivo* dynamometry to the study of the pathomechanics of acute and chronic muscle injury will provide an accurate and reliable means of investigating the causal factors and outcomes of muscle injury. *In vivo* dynamometry provides for control of the biomechanical load (force, range of motion, velocity, number of repetitions) and accurate assessment of the recovery kinetics. The relative non-invasiveness of the procedure provides for longitudinal studies of skeletal muscle injury. The application of this model to the study of skeletal muscle injury will potentially help in elucidating the causal factors of acute and chronic skeletal muscle injury, the pathogenesis of these injuries, and the functional ramifications. Findings from these studies will hopefully aid in the design of better preventative strategies to minimize the occurrence of musculo-skeletal disorders in the workplace.

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