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Medical Education / Method Paper

Effect of Load on *In-situ* Rat Skeletal Muscle Preparation Under Free Loaded and After Loaded Condition

Hanjabam Barun Sharma, P. Jeevitha, Geetanjali Bade, and Simran Kaur*

Department of Physiology,
All India Institute of Medical Sciences,
New Delhi

Abstract

Purpose of the study: The effect of free loaded and after loaded condition in mammalian skeletal muscle contraction can be studied using an in-situ nerve-muscle preparation in rat. The experimental setup mimics the physiological conditions very closely unlike the classical *in vitro* frog nerve-muscle preparation and hence could be considered as an effective practical module to demonstrate the work done in a skeletal muscle in both free loaded and after loaded condition.

Methodology: A simple, feasible and novel practical is designed in which muscle twitch is recorded in an *in-situ* nerve-muscle preparation of an anaesthetized rat under free loaded and after loaded condition at different weights using an isotonic transducer. Work done was calculated under free loaded and after loaded condition and compared.

Main finding: Difference was observed with respect to displacement and work done during free loaded and after loaded condition. Work done was higher in free loaded condition.

Conclusion: We propose a simple and novel practical to demonstrate the effect of free loaded and after loaded condition in muscle twitch for undergraduate teaching in physiology.

Introduction

In response to a single action potential, there occurs a brief contraction followed by relaxation response called muscle twitch in skeletal muscle (1). The muscle twitch can be produced under free loaded or after loaded condition. In case of free loaded conditions, the series elastic component is taut initially when the load stretches the muscle in the relaxed state. This causes increase in both velocity and the height of contraction up to a certain point which is in accordance with Frank Starling's law. Hence, the height of contraction is more in case of free loaded condition as compared to that of after loaded condition (2).

The demonstration of effect of work done by skeletal

***Corresponding author :**

Dr. Simran Kaur, Department of Physiology, AIIMS, New Delhi.
Email: simranaiims@outlook.com

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muscle *in-vitro* under free loaded and after loaded conditions is one of the most important nerve-muscle practical for MBBS teaching (3). Traditionally it is done in gastrocnemius muscle and sciatic nerve preparation of frog in myograph board using isotonic muscle lever and drum-kymograph (2). Unfortunately, the frequent unavailability of frog for dissection, and the lack of accuracy and precision of the traditional set up intrigued us to look into alternatives to demonstrate this phenomenon.

Thus, the current study was conducted with an aim to design an effective and feasible experimental paradigm for estimating work performed by *in-situ* nerve muscle preparation in rat under free loaded and after loaded condition, in order to demonstrate the effect of free load and after load in skeletal muscle to MBBS students with the help of increasing the load.

Materials and Methods

Materials required:

1. Thiopentone sodium (1 g reconstituted to 10 ml or 100 mg/ml) and 1 ml tuberculin syringe.

2. Dissection instruments: scissors, forceps (curved artery forceps and toothed forceps), wooden dissection board or rat dissection tray with four side hooks for tying limb, glass dissecting probes or glass seekers, dental scapula, and bone rongeur.

3. Unyielding-silk thread, warm paraffin wax or mineral oil, 0.9% normal saline, droppers, cotton, gauze pieces, cloth to pick up rat and gloves.

4. Power Lab 26T with Lab Chart 8 software digital data acquisition system, MLT0015 isotonic transducer with a ML221 bridge amp, MLA270 stimulator cable (BNC to Micro-Hooks), MLA40 manipulator with stand (AD Instruments, New South Wales, Australia), rod stand, pulley, pin hook, T connector for pulley (if required), connectors (for T connector and isotonic transducer, if required), calibration weights (5 g, 10g and 15g etc.), and a ruler or measuring tape.

Animal:

A male or female Wistar rat of weight 200-250 g.

Objectives addressed:

1. Define free loaded and after loaded conditions of the muscle.

2. Describe the effect of the above on the latent period, contraction phase, relaxation phase and height of contraction of simple muscle twitch.

3. Explain the mechanism of difference in the work done by the muscle under the two conditions.

Principle:

The effect of free loaded and after loaded condition in isotonic mammalian skeletal muscle contraction can be studied using *in-situ* nerve-muscle preparation in rat which mimics physiological conditions (intact blood supply with constant body temperature) very closely unlike the *in-vitro* nerve-muscle preparation regularly used for physiological practical demonstration. Using isotonic transducer free loading and after loading at different weights was done in sciatic nerve-soleus muscle *in-situ* preparation in an anaesthetized rat, and work done is calculated in each condition from the data collected and compared.

Experimental protocol:

Adult male Wistar rats (body weight 200-250 gm) were obtained from institutional (All India Institute of Medical Sciences, New Delhi, India) central animal facility. The protocol was approved by Institute's Animal Ethics Committee (65/IAEC-1/2018). The detailed procedure is described as follows:

Procedure:

1. Calibration

The calibration of weight or force and displacement

in the isotonic transducer as per the instruction of the manufacturer (4).

2. Animal preparation for the surgery

Acclimatize the rat to the departmental animal house for one day with food (standard rat chow) and water ad libitum. After one day, keep the rat on overnight fast. Anaesthetize with thiopentone sodium injected intraperitoneally with a dose of 50 mg/kg body weight.

3. Surgery and *in-situ* sciatic nerve-soleus muscle preparation

Place the rat on a wooden dissection board and tie all the limbs with thread to four corners. Skin one of the legs from knee and down, dissect the soleus muscle free from surrounding tissue. However, keep the blood supply intake. Take utmost precaution not to damage or put any drag on the blood supply of the muscle.

Place a thread on the distal tendon of the soleus muscle just proximal to its attachment to the calcaneus. Release the distal attachment of the muscle by cutting such that a small piece of calcaneus is still attached to the Achilles tendon. Tie the thread to isotonic transducer with the help of a pulley system if need be so as to keep the transducer in line with muscle-tendon unit, and the pull of the muscle, with the soleus muscle parallel to tibia bone. This ensures physiological movement. Cut the gastrocnemius and plantaris tendons from that of the soleus. Split the fascia between the soleus and the gastrocnemius-plantaris about halfway up the soleus. This should be, however, distal to the blood vessels which supply the belly of the soleus. In this way, the movements of the gastrocnemius and plantaris will not interfere with that of soleus (5). Clamp the ankle and the middle part of tibia to make the leg immobile and stable. The origin of the muscle is immobilized (Fig. 1).

Identify the sciatic nerve and place stimulating electrodes of the PowerLab 26T. Make sure the soleus muscle temperature is around 37°C by pouring pre-warmed 0.9% saline constantly over the

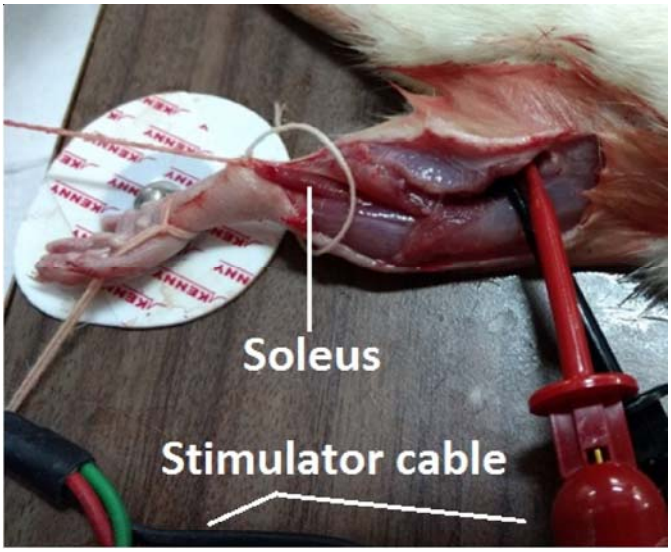

Fig. 1: Experimental set up for nerve (sciatic) – muscle (soleus) *in situ* preparation: The rat was anaesthetized using thiopentone (50 mg/kg) followed by dissection of the soleus muscle and the tendon of the soleus muscle was connected to the isotonic transducer. The sciatic nerve was identified and stimulated using hook stimulating electrodes.

epimysium of the muscle (6). Warmed Krebs-Ringer solution may also be used (5). However, once the setup is done, warmed paraffin or mineral oil may be used instead by pouring it after making a container with the loosened skin of the hind limb being pulled around the sides of the exposed area of interest, so as to minimize evaporative heat loss and distribute heat evenly. A heat lamp may also be used. This is done to keep the temperature as close to body temperature as possible. However, make sure that the core temperature of the animal does not exceed 38°C (7).

Instead of using the *in-situ* sciatic nerve-soleus muscle preparation as above, one may use *in-situ* preparation of gastrocnemius (8) or medial gastrocnemius (6) or extensor digitorum longus (5) or tibialis anterior (9) etc.

4. Recording of muscle twitch under free loaded and after loaded condition

Example of weights used may be 5 g, 10 g and 15 g. The isotonic transducer is set in free loaded and then in after loaded condition at different weights. The sequence of free loaded and after loaded

conditions may be randomized, or recordings are taken in all possible sequences.

The stimulus given is a single square pulse of 1 ms duration starting with 5 V, and gradually increased (e.g., by 0.2V) to find maximal stimulus or maximal voltage. A start delay of 10 ms, maximal repeat rate of 1 Hz and amplifier range of 5 mV may be taken. Different combinations, however, may be used as per the requirement. A 15 ms pulse at 1 Hz, or 0.2 ms pulse, or 50 µs pulse at 0.5V may also be used (5, 7). The sample frequency of 1 kHz or 2 kHz may be used. The above stimulus parameters were chosen after performing and recording muscle twitch using the *in-situ* nerve-muscle preparation.

The recording of muscle twitch in free loaded and after loaded conditions is done at supramaximal stimulus. This is taken higher (eg: 0.5V higher) than the maximal stimulus (5) or double the maximal voltage etc (7). Rest duration of about 1-2 min may be given between two successive stimuli (Fig. 2).

5. Data analysis and work done calculation

Export the data to data pad of LabChart 8 and excel. Record the maximum twitch amplitude or maximal displacement. This is done for free loaded and after loaded conditions at different weights. Calculate the work done in both free loaded and after loaded conditions by using this formula: work done in ergs = weight (g) X maximum displacement (cm) X 981.

6. Sacrificing the animal

After the data collection, the rat is sacrificed with the over dose of anesthetic or neck dislocation while still under anesthesia.

Results

Representative records of displacement by isotonic transducer under free loaded and after loaded conditions at 5 g are shown in Fig. 3 and Fig. 4 respectively. The maximum displacement and hence work done under both the conditions are: (a) free loaded condition: .4290 cm and 2104 ergs, and (b) after loaded condition: .3962 cm and 1943 ergs. The records presented here is for understanding purpose only and the stimulus parameters are different from what is mentioned under materials & methods.

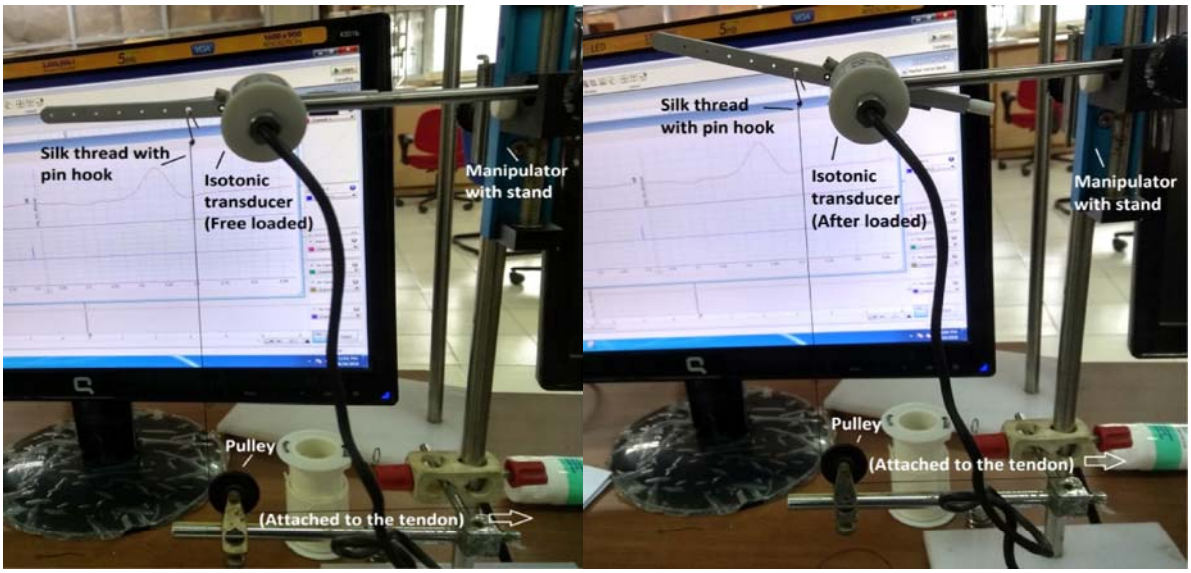

Fig. 2 : Calibration of the isotonic transducer in free loaded (left side) and after loaded condition (right side). Calibration of the isotonic transducer was done by hanging a known weight in the lever hole where the tissue is to attach. Adjust the counterweight screw until the lever arm is balanced. The weight is removed (lever will move upwards) and the muscle is attached to the same hole used for calibration (lever moves down after this). The tissue is thus set at the load equal to the calibration weight (5, 10, 15 kg) used for the experiment.

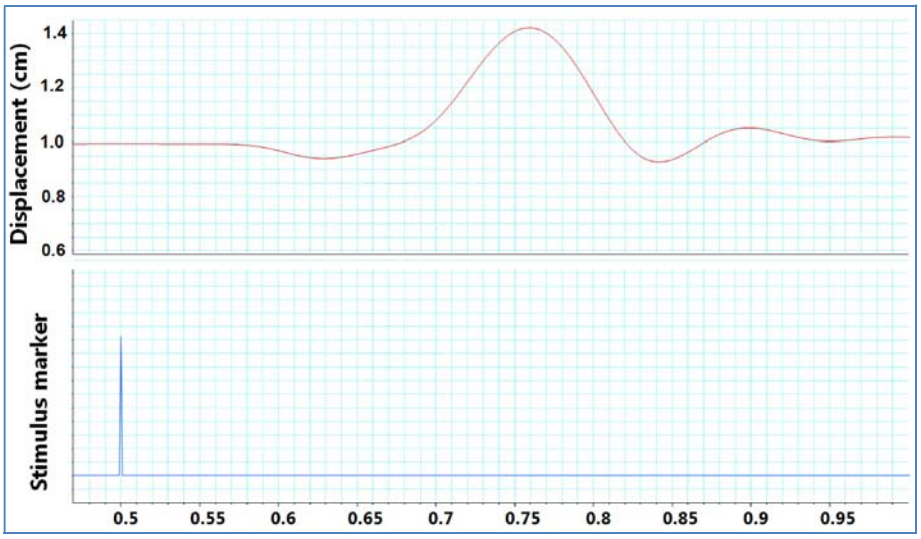

Fig. 3 : Representative graph of the soleus *in-situ* nerve muscle preparation in the rat under free loaded condition: Isotonic transducer was used at 5 g under free loaded condition (stimulus parameter: 16V for 0.1 s. X-axis shows the time recording. Y-axis shows the displacement recorded (.4290cm)

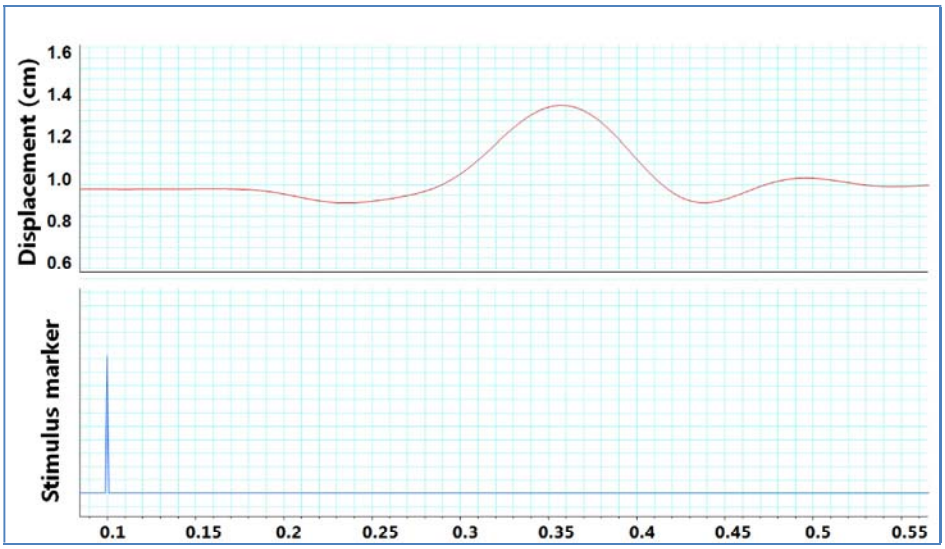

Fig. 4 : Representative graph of the soleus *in-situ* nerve muscle preparation in the rat under free loaded condition: Isotonic transducer was used at 5 g under after loaded condition (stimulus parameter: 16V for 0.1 s. X-axis shows the time recording. Y-axis shows the displacement recorded (.3962 cm)

Discussion

The paper presents an innovative practical teaching experiment to demonstrate the estimation of work performed by skeletal muscle of a rat *in-situ*, which in our case is soleus, under free loaded and after loaded conditions at different weights. Using this practical demonstration, the student will be able to understand easily the important concept of free loading and after loading, and hence the Starling's law. The weights and contraction velocity relationship under free loaded and after loaded conditions may also be studied. The effect of free load and after load on characteristics of muscle twitch like latent period, contraction and relaxation period can also be demonstrated.

The assessment of learning of this demonstration practical may be done as a part of objectively

structured practical examination (OSPE) with question stations based on graphs obtained under free loaded and after loaded condition, and the underlying physiological mechanisms.

Further extension of the practical may be done for post graduate teaching by including the component to study the effect of successive stimuli, genesis of post tetanic potentiation, staircase effect, tetanus and phenomenon of fatigue under free loaded and after loaded conditions. Additional measure like electromyography (EMG), blood flow or biochemical analysis may be included to assess maintenance or change in membrane excitability, motor unit firing & recruitment; blood flow or depletion of nutrient and energy supply etc. The effect of training, disuse, injury, drugs or disease can also be studied (6).

Acknowledgements

None

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