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# Spectrophotometric Changes in Glycogen Content of Gastrocnemius and Soleus Muscles in Response to Achilles Tenotomy in Rat

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## Abstract

**Background** The glycogen content of individual muscle fibers varies according to their types as well as in response to workload changes. The effects of tenotomy on this glycogen content in skeletal muscles of the rat leg have long been studied, however, such effects on the contralateral limb was not detailed in most of these studies.

**Objective** To investigate the effect of tenotomy of the Achilles tendon on the glycogen content of gastrocnemius and soleus muscles fibers in both the tenotomized and contralateral limbs.

**Methods** Thirty adult male rats (*Rattus rattus norvegicus albinus*) divided into non-tenotomized (control) and tenotomized (experimental) groups. The Achilles tendon of the right hind limb was incised and left to heal for 2 weeks. Animals then were sacrificed and cross sections of the right and left gastrocnemius and soleus muscles were examined spectrophotometrically for the mean optical density of PAS stain.

**Results** A significant decrease in the glycogen content of the tenotomized and contralateral limbs of gastrocnemius muscle compared with the control group. Similar significant decrease was found for soleus muscle in tenotomized but not the contralateral limb of the experimental group compared with the control group.

**Conclusions** The disuse atrophy in the tenotomized limb causes decrease in the glycogen content of gastrocnemius and soleus muscle fibers. However, the change in glycogen content seems to affect the fast gastrocnemius rather than the slow soleus musclein the contralateral limb probably because the mechanical load on gastrocnemius during muscle contraction following tenotomy is higher than that exerted on soleus muscle.

**Key words** Skeletal muscle, Gastrocnemius, Soleus, Glycogen, Spectrophotometry.

## Introduction

Skeletal muscle is made up of different fiber types, in which glycolysis for energy production takes place. Three types of skeletal muscle fibers can be identified by color: red, white, and intermediate. Red fibers are small and contain large amounts of myoglobin. Intermediate fibers are of medium size with high myoglobin content and large amounts of glycogen. White fibers are large and contain less myoglobin and they store a considerable amount of glycogen [(1)]. Skeletal muscle fibers specialize in the transformation of chemical energy into mechanical events, i.e. force production. Chemical energy is stored in glycogen particles, which constitute about 0.5-7 % of muscle weight [(2)].

The proportion of each type of skeletal muscle fibers varies between muscles, and the classification of fiber types in muscle biopsies has clinical significance for the diagnosis of muscle diseases or myopathies [(3)]. The same

fiber type with identical metabolic properties may have different size in different muscles. Accordingly, slow muscle fibers, for example, in the rat soleus are bigger than slow fibers present in rat fast muscles. Thus both fiber type and muscle of origin are relevant for fiber size [4].

The relative glycogen content of muscle fibers can be estimated histochemically with the periodic acid Schiff's (PAS) reaction [5]. In 1979, Halkjaer and Ingemann described identical concentrations of PAS stain (color) in histological sections of the same human muscle in both cryostat and paraffin-embedded sections [6]. The histochemical assessment of the glycogen content in various types of muscle fibers is usually carried out by subjective rating of the PAS staining intensity in animals [7]; in addition, this subjective rating has been also used for human muscles [8]. However, a good linear relationship was found between computerized image analysis of the optical density of PAS stained sections and biochemical assessment of glycogen content of single fibers [9].

The rat soleus, slow-twitch muscle involved in maintaining posture, contains a high percentage of slow type I fibers [10]. In contrast, both heads of gastrocnemius muscle have a majority of fast type II fibers [11].Studies investigating the effect of tenotomy on glycogen content of slow and fast skeletal muscle fibers indicate that glycogen content shows no changes within the first three hours [12], then it significantly increases 12 hours after tenotomy, with the contralateral limb serving as a control [13]. Glycogen has also been shown to increase when the tenotomized limb was compared to a non-tenotomy control. After that peak, glycogen drops to lower levels and maintains this drop [14]. To present, changes in glycogen content of the contralateral limb in comparison to the non-tenotomy control have not been investigatedyet.

The purpose of this workis to use the PAS stain for studying the effect of sharp cut injury (tenotomy) of the Achilles tendon on the glycogen content of gastrocnemius and soleus muscles fibers in both the tenotomized and contralateral limbs.

## Methods

### *Animal preparation and sampling*

The work was done during the period from January to March 2012; at the Anatomy Department of Al-Nahrain University / College of Medicine.A sample of 30 adult male rats (*Rattus rattus norvegicus albinus*) aged 3-6 months with 300±50 g body weight were chosen. Animals were divided into two groups: non-tenotomized (control) and tenotomized (experimental) groups (15 rats in each group). Animals were housed 1 per cage, given access to drinking water, and fed standard diet pellets. Cages were 60 cm length by 30 cm width and 30 cm height to ensure providing an environment suitable for reasonable activity.

Open ether anesthesia was used in all surgical procedures. Animals were anesthetized with ether-impregnated cotton-wool in airtight jar for 2-3 minutes prior to tenotomy. The animal was put in prone position and the right foot was dorsi-flexed for the Achilles tendon to be prominent. A transverse incision was made at the lower end of the posterior aspect of the leg just above the calcaneus to cut the Achilles tendon [15]. The gastrocnemius and soleus muscles got contracted up the leg. Skin wound was sprayed by Inibsaplastospray and the cut tendon was left to heal spontaneously. Upon recovery from anesthesia, the right foot was in dorsiflexion.

The control and experimental groups were sacrificed after ether-induced deep anesthesia at the end of the experiment (2 weeks after tenotomy). Both right and left gastrocnemius and soleus muscles from the control group, and the right (tenotomized), and the left (contralateral) gastrocnemius and soleus muscles from the experimental group were excised. Whole muscles were fixed immediately in 10% formalin for further tissue processing. Paraffin blocks were prepared and sectioned at 10µm thickness.

### *Staining*

After slides dewaxing and hydration, sections were oxidized in 1% periodic acid for 10 minutes and then washed well in distilled water for about

30 seconds. Slides were then treated with Schiff reagent for 20 minutes at 38°C, dehydrated in graded alcohol (50%, 70%, 80%, 90% and 99%) 1 minute for each, cleared in xylene (2-3 minutes), and mounted in Eukitt. Control sections were pre-incubated in1% amylase (30 minutes, at 37°C) before oxidation in periodic acid [16].

***Quantification of PAS staining intensity***

A Poly specmicro spectrophotometer system supplied from Reichert-Jung was used in this study. Muscle samples from soleus and gastrocnemius were labeledas control and experimental groups, the latter was subdivided into tenotomized and contralateral limbs. Using a systemic random selection of 5 fields per section, ten muscle fibers from each field were selected; they were identified subjectively asType I, Intermediate and Type II in order of increasing size (perimeter) for gastrocnemius muscle,and Type I and Intermediate for soleus muscle, which is in agreement withthe previous results of Al-Kaabi [17].

In order to measure the mean optical density of muscle fibers stained with PAS stain, the value of the isobestic wave length or wavelength of maximum absorbance was entered as 510 nm [18]. The measuring diaphragm was fitted so that to include most of the inside of the selected fibers. The average absorbance of each muscle fiber type was calculated, representing the optical density of PAS stain in each type. ANOVA and paired T-tests between the mean optical density values of muscle fiber types in the control group, tenotomizedand contralateral limb of the experimental groupwere performed using Microsoft Excel 2010 statistical software.

## Results

Cross-sections of controlgroup of gastrocnemius and soleus muscles, tenotomy and contralateral limbs of the experimental group of both muscles are shown in figure 1. The control group showed the mosaic pattern of staining intensity resulting from the different content of glycogen within the different muscle fiber types. Both gastrocnemius and soleus muscles in the tenotomy group revealed decreased colorintensity with almost homogeneous staining pattern. In the contralateral group, only gastrocnemius muscle showed marked decrease in the staining intensity as compared to that of the control group.

Spectrophotometric measurements of the mean optical density of PAS stain in gastrocnemius and soleus muscles are shown in figures2 and 3. For gastrocnemius muscle, ANOVA showed that there was significant decrease ($P$ <0.05) in the mean optical density of the tenotomy and contralateral limbs of the experimental groupversus the control group. The decrease was also significant ($P$ <0.05) when comparing the contralateral limb versus the tenotomy limb of the experimental group.

For soleus muscle, ANOVA revealed significant decrease ($P$ <0.05) in the mean optical density between the tenotomy limb and the control group, along with that between the tenotomy limb and the contralateral one. However, the decrease was not significant when comparing the contralateral limb of the experimental group versus thecontrol group.

## Discussion

When the tenotomy limb was compared with the control group, the mean optical density of PAS stain decreased significantly for gastrocnemius and soleus muscles, reflecting the decrease in glycogen content of both muscles. This decrease may be due to the effect of tenotomy itself [14] or can be due to a higher workload thrust upon both muscles as a result of muscle shortening. Studies suggest that the effect of tenotomy or disuse atrophy is a more acceptable explanation [20] since the variability of glycogen stores in response to muscle contractions is at its minimum in the second week after tenotomy.

Results of the mean optical density of PAS stain in the contralateral limb of the experimental group were different. The significant decrease in the optical density of the contralateral limb compared with control group in gastrocnemius muscle suggests that mechanical load on this muscle during muscle contraction (weight

bearing activity and locomotion) following tenotomy is higher than that exerted on soleus muscle. Tenotomy causes limping and changes the weight bearing activity and locomotion in both limbs. The change in these parameters seems to affect the fast gastrocnemius muscle rather than the slow soleus muscle.

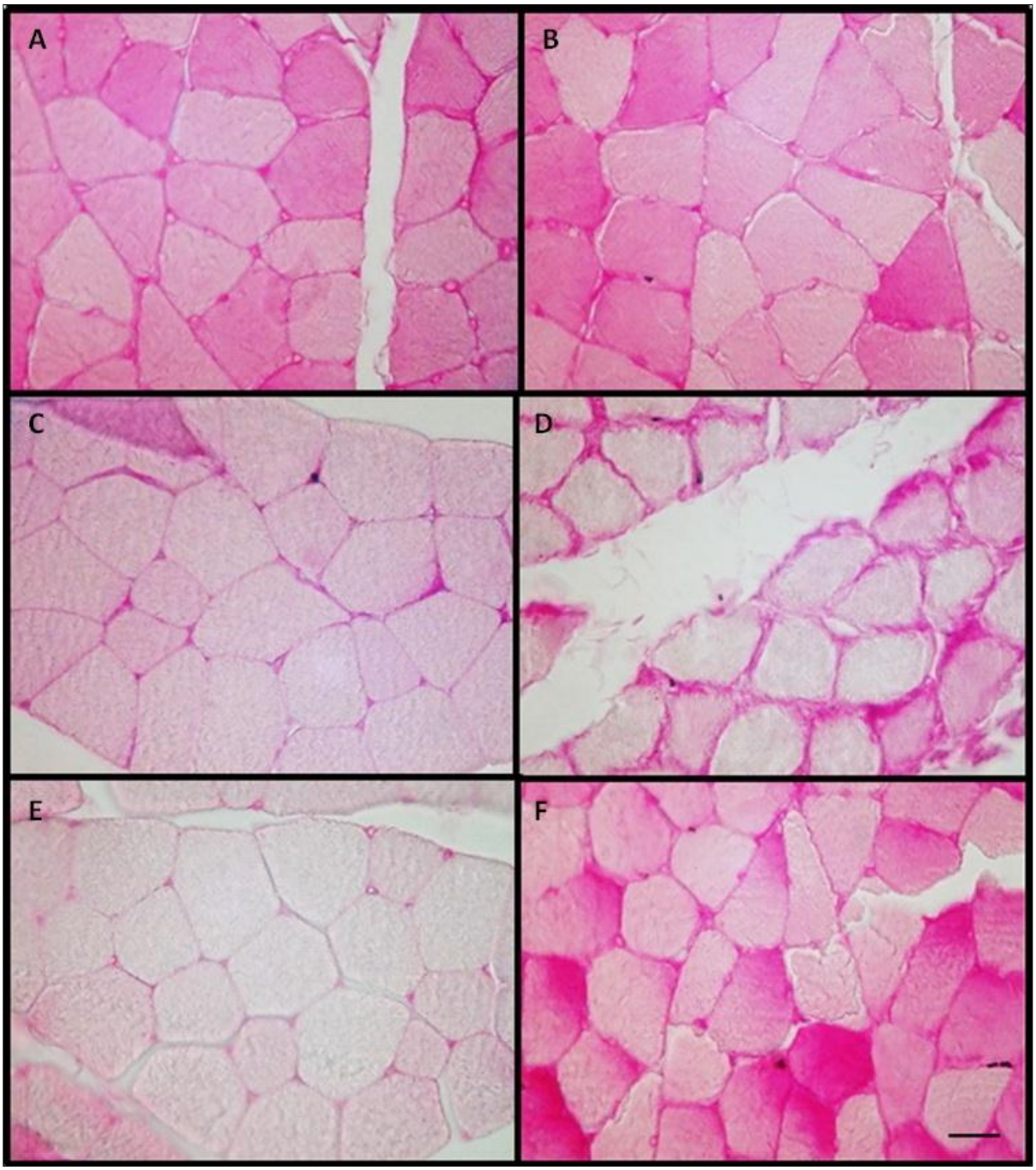


**Fig. 1. Cross-sections of rat muscles stained with PAS stain. (A) Gastrocnemius and (B) soleus of the control group. (C) Gastrocnemius and (D) soleus of the tenotomy limbs. (E) Gastrocnemius and (F) soleus of the contralateral limbs in the experimental group. Both C and D show clear decrease in the intensity of PAS stain. A similar observation is evident in E, however, the PAS staining intensity seems to be unaffected in F. 400 X. Bar = 50 µm**

These results are in agreement with studies on workload changes [21]. It seems thattenotomy caused the contralateral limb to depend on gastrocnemius to a greater extent than soleus muscle for its weight bearing activity as well as locomotion, an assumption that might be supported by the fact that gastrocnemius contains abundance of fast type II fibers while soleus contains predominantly intermediate fibers with absence of type II fibers [17]. These data suggest that the contralateral limb cannot be used as a control, a trend which can be seen in many previous studies [12].

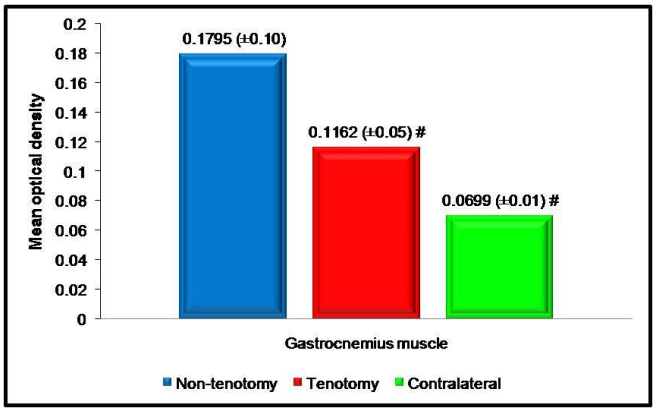


**Fig. 2. The values of mean optical density (±SD) of gastrocnemius muscle for control group, tenotomy and contralateral limbs of experimental group.**

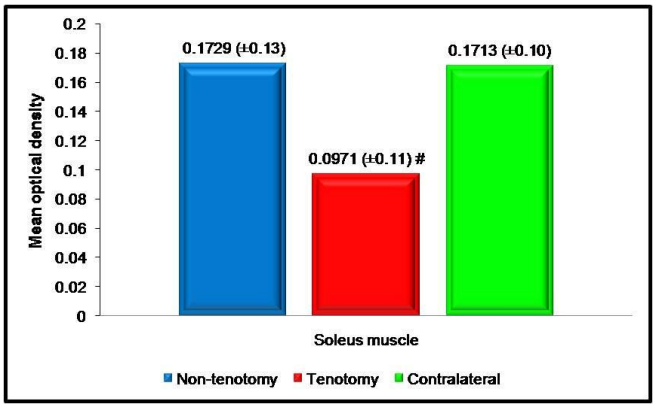


**Fig. 3. The values of mean optical density (±SD) of soleus muscle for control group, tenotomy and contralateral limbs of experimental group.**

A change in the workload on muscles of both sides will always ensue after tenotomytill complete healing of the tendon [21]. Limpingcauses redistribution of the workload exerted on the contralateral limb, a change that is expected to vary with time in accordance with the healing state of the tendon and the condition of the affected muscles. Therefore, it is recommended to follow up the changes in the workload exerted on the contralateral muscles at different times after tenotomy.

So we conclude from this study that thedisuse atrophy in the tenotomized limb causes decrease in the glycogen content of gastrocnemius and soleus muscle fibers. However, the change in glycogen content seems to affect the fast gastrocnemius rather than the slow soleus muscle in the contralateral limb probably because the mechanical load on gastrocnemius during muscle contraction following tenotomy is higher than that exerted on soleus muscle.

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