

How to Cite:

Jawad, A. I., & Alalwany, E. A. H. (2022). Histomorphometric, histochemical, comparative study of skeletal muscle in male and female albino rat. *International Journal of Health Sciences*, 6(S10), 206–213. <https://doi.org/10.53730/ijhs.v6nS10.13381>

Histomorphometric, histochemical, comparative study of skeletal muscle in male and female albino rat

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Abstract--Histomorphological and histochemical study, by light microscopy, was undertaken on the skeletal muscle of the albino rat different sex (male and female). The body weight and muscle (biceps barchii in arm and biceps feomris in thigh), were weighted, measured and stained with Hematoxylin and eosin, Masson's Trichrome stain. The results revealed there's significant differences in weight of body, weight of skeletal muscle (biceps barchii in arm and biceps feomris in thigh), and diameter of muscle bundle, was increased in male rat to the female and there is significant difference between them . By the other hand the results of number of muscle fiber measuring of skeletal muscle in both sexes showed no significant changes.

Keywords--rat, histomorphological, histochemical, biceps barchii, biceps feomris.

Introduction

Muscle tissue is the most common type of tissue in the body, making up about 40% of total body weight. It is made up of fibers, which are long, elongated cells that develop from the mesoderm layer during embryonic development. Because of its ability to contract and relax, muscle tissue is involved in all of the body's movements. The blood arteries are dispersed between the muscle fibers to both nourish the tissue and control its function (Zaytoun. 2008). Using phenotypic and functional traits, it is possible to discriminate between three different forms of muscle tissue in mammals. Additionally, each type of structural adaptation corresponds to a physiological function: 1. Smooth muscle is a slow-contracting, involuntary muscle made composed of clusters of spindle cells without transverse

patterns. (Al-Hamoud and Walid,2003),2. Cardiac muscle is a transversely striated muscle made up of parallel, elongated, and branched cells. Intercalated discs are seen at the points where the fiber ends of the muscle fibers come into contact with these cells. The cross-striated skeletal muscles contract quickly and forcefully. A morphologically and functionally dynamic tissue (Leiter,2009), Because myoglobin pigment is found in muscle fibers and there is an excess of blood supply to the tissue, skeletal muscles appear pink in their fresh state. There are several variations in the kind of skeletal muscles, characterized by red and white muscles. (Gali 1991) Skeletal muscle is made up of several muscle fibers with various histological and physiological characteristics (Barbara, and Reet ,2000). He demonstrated that myofibers, which are lengthy cylindrical structures enclosed by a membrane known as the Sarcolemma and an Overlying Basal Lamina, are the cellular units that make up skeletal muscle fibers. when the muscle fibers have several flat nuclei encircling the periphery of the fiber (Mahood ,2008.).

Materials and Methods

Animals

Forty healthy adult albino rats regardless to their sex of twenty adult male and twenty adult female based on the differences in their age and weight were used in the present study. . *Rats were bought from specific market at Babylon province from the local suppliers. were housed in individual cages, and they were fed as well and giving them water before their euthanasia and dissection.* Rats were divided into two parts, the first part including ten rats of each species focused on the morphological part. The second one also including ten rats of each species focused on the histometrical and histochemical parts.

Preparation of Specimens

Rats were weighted by digital balance, The Skeletal muscles were extracted by stripping off the skin of rats from the front and hind limbs and isolating the required muscles from the front and hind limbs. Then the required muscles were weighted with a sensitive scale after removing the surrounding connective and fatty tissues. The skeletal muscle was identified and photographed *in situ* using digital camera.

Morphological Study

For gross observation twenty rats were used, which divided into two equal parts, ten males, ten females, which were represented the study of position , shape, color The parameters of this part. Body weight, Weight of muscle. All measurements mentioned above were listed in tables. The weights were measured in grams by using sensitive electron balance.

Histological Study

Twenty Rats were used for histological observation (ten rats of each sex). The specimens performed according to (Suvarna *et al.*,2012).

Staining

The following stains were utilized in the current study

- Harris Hematoxylin and Eosin Stain: These are naturally occurring substances that have been employed throughout the history of histopathology (Titford, 2009).
- Masson's Trichrome stain : For the staining the connective tissue fibers. According to historical analysis of the usage of different stains in histology, pathologists were particularly drawn to stains that produced multicolored findings on tissue specimens. Trichrome stains were created as a result of this necessity (Shostak, 2013).

Statistical Analysis

the data was analyzed performed using SPSS (version 16.0). (Field, 2013). The mean values \pm standard error are used to represent all numerical findings . Ronald Fisher, a statistician, has used an ANOVA to evaluate the statistical significance of comparisons. The threshold for significance was established at ($p < 0.05$).

Results and Discussions

Anatomical study

The relationship of body weight and muscle weight

The anatomical results showed that the body weight and relationship with sex in laboratory rats showed that the average body weight of the rats was (490.600 ± 1.364 g) in males, while it was in the female (449.800 ± 1.463 g .) respectively (Tab. 1). This result was similar to the finding of (Ethun,2016)) who mentions that the energy metabolism and vulnerability of males and females to pathophysiological diseases, such as type II diabetes and obesity, are different. Body composition, lipid metabolism, and glucose regulation distinctions between sexes might change over time or as a result of a person's lifestyle. Our findings about changes in male and female body weight concur with those in ((Bowman et al., 2006).

Table 1
Show the body weight of male and female albino rats

Sex	Body weight Me \pm SE
Male	490.600 ± 1.364 *
Female	449.800 ± 1.463 *

The numbers represent the mean \pm standard error.

* = Denotes that a significant difference was found between male and female rats at the level ($P < 0.05$).

Measurement of Arm weight of male and female albino rats

The average weight of the biceps muscle in the anterior limb of rats increased significantly in males (0.954 ± 0.002 g), while in the female (0.576 ± 0.021 g). (Tab. 2)

Table 2
Show the arm weight of male and female albino rats

Sex	Thigh weight Me \pm Se
Male	0.943 ± 0.0134 *
Female	0.905 ± 0.0094 *

The numbers represent the mean \pm standard error.

* = Denotes that a significant difference was found between male and female rats at the level ($P < 0.05$).

According to the results of this study, (Enad ,2004) mention that the anatomy of the muscle is clearly observed The origin composed of short head located at the medial side on deep fascia of medial part of forearm that connect with coracoid process of scapula and the long head located on the lateral side of biceps brachii that connected with supraglenoid tuberosity. The insertion that connected with radial tuberosity and bicipital aponeurosis into deep fascia on medial part of forearm. Mehta and Agnew's, (2010) indicated that the muscles of the upper limbs tend to differ more between the sexes than those of the lower ones. However, significant overlap has also been demonstrated between the sexes.

Measurement of Thigh weight of male and female albino rats

The average weight of the biceps femoris muscle in the posterior limb of rats increased significantly in males (0.943 ± 0.0134 g), while in female (0.905 ± 0.0094 g) , (Table. 3).

Table 3
Show the Thigh weight of male and female albino rats

Sex	Arm Weight Me \pm Se
Male	0.954 ± 0.002 *
Female	0.576 ± 0.0216

The numbers represent the mean \pm standard error.

* = Denotes that a significant difference was found between male and female rats at the level ($P < 0.05$).

biceps the femoris is a two-part muscle that resembles a spindle. The long head and the short head are its two heads. The two heads originate from various locations and are innervated by several nerves, yet they connect distally and insert at the same spot. This finding is consistent with (Blackburn et al., 2009), which shows that the biceps femoris muscle has two heads of origin and is a powerful flexor of the knee joint. This muscle also stretches the hip joint, rotates the leg laterally, and has been demonstrated to be more active in knees with anterior cruciate ligament deficiencies.

Histological study

- **Skeletal muscle:**
Numerous fascicles, or bundles of muscle fibers, can be found in skeletal muscle. A fascial connective tissue layer surrounds each muscle fiber and fiber individually (Fig. 1).
Muscle bundles, connective tissue, muscle fiber, perimysium, nerve supply, blood vessels, and muscle spindles make up the female albino rat's thigh muscle (Fig. 2,3,4).
- **Diameter of muscle bundle:**
The histological results revealed the diameters of the muscle bundles of arm the biceps brachii muscle in the anterior limbs of male rats increased more than that female showed that ($0.398 \pm 0.010 \text{ } \mu\text{m}$) , ($0.298 \pm 0.006 \text{ } \mu\text{m}$) , respectively . As the table (4). While in the biceps femoris it was the diameter of bundle in male increased more than that female showed that ($0.406 \pm 0.009 \text{ } \mu\text{m}$) , ($0.270 \pm 0.007 \text{ } \mu\text{m}$), respectively.(Fig.1,2).

Table 4
Diameter of muscle bundle in both sex rats

Sex	Arm Me \pm Se μm	Thigh Me \pm Se μm
Male	$0.398 \pm 0.010^*$	$0.406 \pm 0.009^*$
Female	$0.298 \pm 0.006^*$	$0.270 \pm 0.007^*$

The numbers represent the mean \pm standard error.

* = Denotes that a significant difference was found between male and female rats at the level ($P < 0.05$).

Hills *et al.* (2010) observed that changes in the relative proportions of water, muscle, fat, and bone occur as a result of hormone production throughout puberty. Under the influence of testosterone, males often experience a large increase in bone and muscular growth as well as a concurrent loss of fat in the limbs.

Number of muscle fiber

The histological results revealed that the number of the muscle fiber and their relationship with sex in the biceps brachii muscle in the anterior limbs of rats showed that the average number of the males ($38.000 \pm 0.837 \text{ } \mu\text{m}$) , while the

number of muscle fiber of female ($38.200 \pm 0.583 \mu\text{m}$). There is not a statistically significant difference ($P = < 0.05$). As the table (5). While in the biceps femoris it was the number of muscle fiber ($43.200 \pm 0.663 \mu\text{m}$), while in female ($42.000 \pm 1.255 \mu\text{m}$). There is not a statistically significant difference ($P = < 0.05$).Table (5).

Table 5
The number of muscle fiber in skeletal muscle in both sex of rat

Sex	Arm Me \pm Se μm	Thigh Me \pm Se μm
Male	43.000 ± 0.663	38.000 ± 0.837
Female	42.000 ± 1.225	38.200 ± 0.583

The numbers represent the mean \pm standard error.

* = There was not a statistically significant difference between male and female rat at level ($P < 0.05$).

This result agreement with Heather, (2022) and Tanganelli *et al.*, (2021) who mentioned that Skeletal muscle has a fixed number of fibers, and muscle mass is increased by normal growth. Fiber number increases were caused by circumferential and longitudinal growth because of the expansion or addition of myofibrils and the addition of sarcomeres.

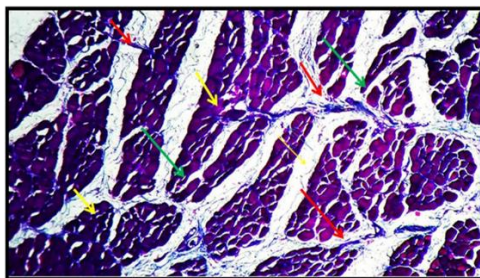


Figure 1. Cross histological section of female thigh shows :Muscle bundles (yellow arrow),connective tissue (red arrow) ,muscle fiber (green arrow) . Masson stain . 20X

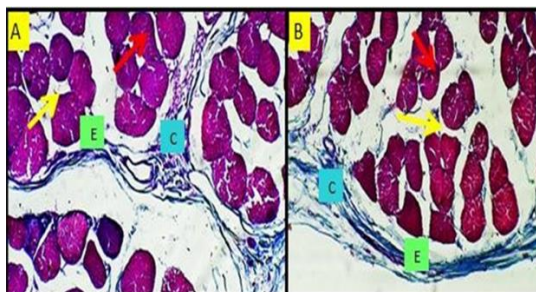


Figure 2. A & B. Cross histological section of male arm shows :perimysium (yellow arrow) , endomysium (red arrow), epimysium (EP) , connective tissue(CT). Masson stain .20X

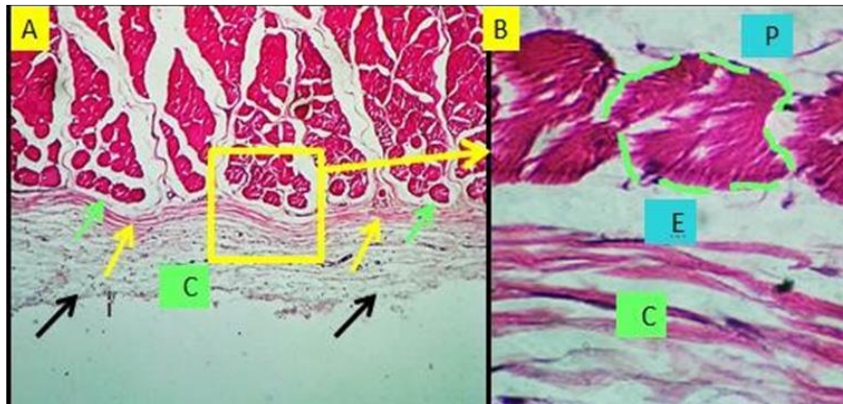


Figure 3. cross section of male thigh shows: A. connective tissue (yellow arrow), capsule of muscle (black arrow), epimysium (green arrow). H& E stain. 10X. B. Magnified section muscle fiber (circle green), perimysium (PR), epimysium (EP), connective tissue (CT).H&E .40X



Figure 4. Cross histological section of female arm shows: Blood and nerve supply of muscle (black circle), connective tissue (blue arrow), capsule of muscle (A) muscle bundle (yellow circle) perimysium(black arrow) .H&E stain. 4X

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