Original Articles/นิพนธ์ต้นฉบับ

Skeletal muscle studying in Non-skeletal Disease Thai Population: Cadaveric cases.

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Abstract

Objectives: To present normative data on muscle fiber type proportion and mean fiber size of the biceps brachii and vastus lateralis muscles in non-skeletal muscle disease Thai population.

Methods: Muscle samples for each of the left biceps brachii (n=20) and the left vastus lateralis (n=20) muscle were taken from 20 cadavers (14 males and 6 females) within 18 to 36 hours after sudden accidental death or who had died after a short acute illness without evidence of neuromuscular disease. Three fiber types (typeI, type IIA, and typeIIB) were classified using myofibrillar ATPase histochemistry. Fiber type proportion and the lesser diameter of muscle fiber type I, type IIA and type IIB was measured. The mean lesser diameter of each fibers was analyzed statistically comparing between males and females, between age group less than 50 years old (<50 yrs) and age group 50 years old or more (≥50 yrs), and between biceps brachii and vastus lateralis muscles.

Results: The fiber type percentage of biceps brachii and vastus lateralis muscle showed predominance of type II fiber and no significance differences between type IIA and type IIB proportions. There were no significant difference in the percentage of each type between males and females. The mean lesser fiber diameter of biceps brachii and vastus lateralis muscles in our study was in normal range established by previous standard reference. Type I fibers were larger than type II fibers, and type IIA fibers were larger than type IIB fibers in males and females. In vastus lateralis muscle, age group less than 50 years old (<50yrs) had larger fiber size than age group 50 years old or more (≥50 yrs) in all type of muscle fibers.

Conclusion: There were no gender difference in the fiber size and fiber proportion in our study. The mean lesser fiber diameter of type I, type IIA and type IIB in non-skeletal muscle disease Thai population was within the interval established from the data published by previously standard reference. Aging had influence on muscle fiber by decrease fiber size in all three types in vastus lateralis muscle.

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Introduction

In order to diagnose various muscle diseases, muscle biopsy is very necessary. The standard for evaluation of muscle biopsy by light microscope routinely use both histological and histochemical methods[1]. Histochemical methods have a role in determine various type of muscle fiber, which is a key of diagnosis. The biceps brachii and vastus lateralis muscles are the standard muscle biopsied by international agreement. Because there are well clear of major vessels and nerves and are often affected in generalized and proximal myopathies[1-5].

Assessment of muscle fiber size and type proportion is fundamental to interpretation of pathological changes seen in muscle biopsy. Fiber size is regulated and influenced by innervation, a number of growth factors such as hormones, and the amount of work that the muscle is subjected to[1]. Excessive load on muscle induces an increase in fiber size while disuse causes a decrease in size. Different gender, age, muscle group and even fiber type (type I, type IIA, type IIB) have different normative fiber size data[1,3,6,7]. The mean fiber diameter is established by gender, each type of fiber (type I, type IIA, type IIB) and muscle group for use as reference data in evaluation of muscle biopsy. Most standard references in the literature are obtained from western populations. The published data that many workers rely on was made by Brooke and Engel, in 1969[8]. For Non-skeletal disease Thai population, the study in normal muscle fiber size and type proportion is the first study in Thailand.

The purpose of our study was to collect normative data on muscle fiber type proportion (percentage of each fiber type) and fiber size of the biceps brachii and vastus lateralis muscles by obtained from 20 cases of Thai adult cadavers without history of neuromuscular disorder and to establish these data for standard reference values in Thai population. In addition to the morphomatric data, we also interested in difference of fiber size between genders and influence of aging on the fiber size.

Materials and methods

Muscle samples for each of the left biceps brachii and the left vastus lateralis muscles were taken from 20 cadavers within 18 to 36 hours after sudden death[9]. There were 14 males and 6 females (Figure

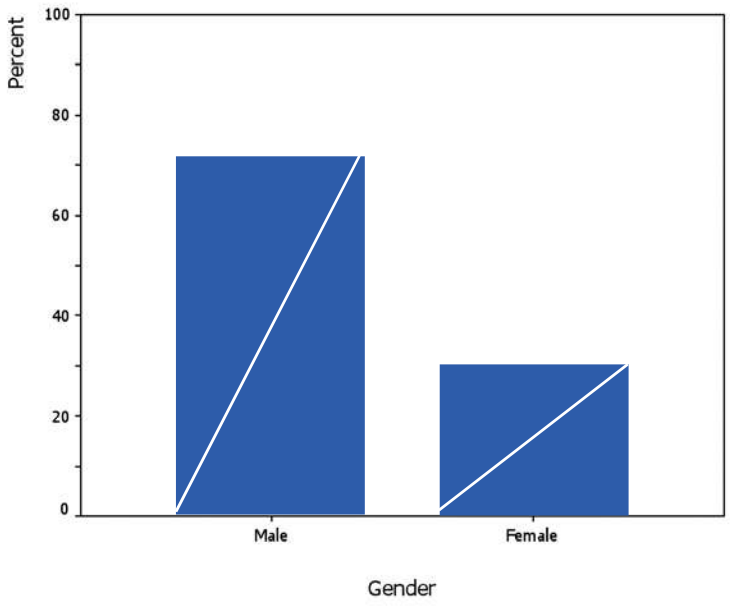

Fig.1 *Percentage of number of male (n=14) and female (n=6) subjects in our study*

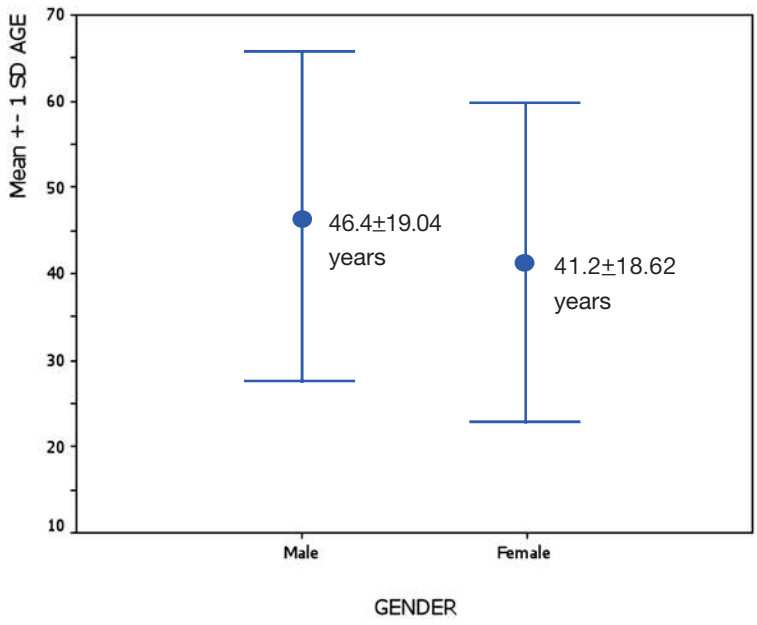

Fig.2 *Mean and SD of male (n=14) and female (n=6) age*

1.), whose aged range from 15-88 years (Figure 2. mean male age: 46.4±19.04 years; mean female age: 41.2±18.62 years). Each individual had suffered a sudden accidental death or who had died after a short acute illness. None had a history of neuromuscular disease, nor was there evidence of pathological abnormalities at the post-mortem examination.

All muscle biopsies were manipulated by the open biopsy technique and immediately frozen in isopentane cooled with liquid nitrogen and oriented in order to obtain transverse sections.

The samples were stored at -70°C until further processing. Frozen sections of 10 μm were cut in a cryostat at -20°C transversely against the longitudinal axis of the muscle fibers[1-5]. Each sections were stained with hematoxylin & eosin, and submitted to histochemical reaction to demonstrate myofibrillar adenosine triphosphatase (ATPase) activity incubated at pH 4.3, 4.6, and 9.4, for identification of fiber types. Additional staining reactions included periodic acid Schiff (PAS), periodic acid Schiff-diastase (PASD), modified gomori trichrome, and succinic dehydrogenase.

In ATPase stained sections, images of a representative parts of the sections were taken at 100 x magnification. Several images for each case were imported into the image analysis program (Image-Pro Plus 6.1 software). The fibers were counted on the pH 4.6 preparation as type I, type IIA, and type IIB for percentages of each fiber type in each muscle sample (Figure 3.)

In order to measure these fibers, a reference ruler was constructed from photograph, also at 100 x magnification, of a 100 μm intervals. Only areas without artifacts and distinct cell borders were measured for their lesser diameter of individual fibers types; type I, type IIA, and type IIB. The lesser diameter was the greatest distance across the lesser aspect of the muscle fiber[1]. At least 100 fibers of each type were measured[1]. A mean fiber diameter and standard deviation is calculated. The data of biceps brachii and vastus lateralis muscles were grouped according to sex and age group (under 50 years old and had 50 years old or more).

This study was approved by the Ethical Clearance of Committee on Human Right Related to

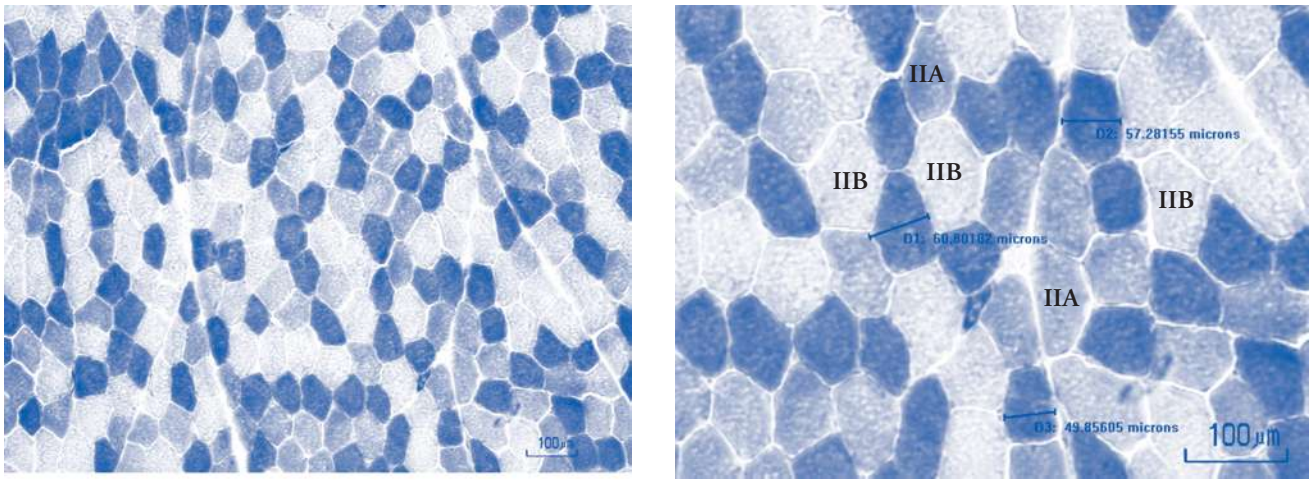

Fig.3 *Cross-section of muscle sample taken from biceps brachii muscle demonstrating the three fiber types, type I (dark stain), type IIA (light stain) and IIB (intermediate stain), in a mosaic pattern. Myofibrillar ATPase at pH 4.6, Bar=100 µm*

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Statistical analysis

The statistical package SPSS was utilized for all statistical analyses. Descriptive statistics were used to derive mean±SD for fiber size (lesser fiber diameter). The statistical comparisons of mean fiber diameter between data of males and females and between data of age group less than 50 years old and age group equals 50 years or more were made using Student's t -test. Differences were considered significant at $p<0.001$

Result

The average percentage of type I, IIA, and IIB muscle fibers from biceps brachii muscle and vastus lateralis muscle in males and females are presented in Table 1. The proportion of fiber types was almost identical for males and females and for biceps brachii muscle and vastus lateralis muscle.

Table 1 Percentage of fiber type proportion of biceps brachii and vastus lateralis muscle in males (n=14) and females (n=6)

	Type I	Type IIA	Type IIB
Biceps brachii			
Male (n=14)	35.57%	31.72%	32.71%
Female (n=6)	31.76%	31.42%	36.82%
Vastus lateralis			
Male (n=14)	36.60%	30.88%	32.52%
Female (n=6)	34.83%	30.63%	34.54%

Table 2 Mean fiber diameter of biceps brachii and vastus lateralis muscles in males(n=14) and females (n=6)

Diameter	Type I Mean (SD) (μm)	Type IIA Mean (SD) (μm)	Type IIB Mean (SD) (μm)
Biceps brachii			
Male (n=14)	63.91 (12.28)	62.37 (10.47)	59.87 (9.78)
Female (n=6)	60.85 (10.68)	58.75 (8.38)	55.86 (8.93)
Vastus lateralis			
Male (n=14)	65.65 (14.04)	63.90 (12.25)	61.67 (12.08)
Female (n=6)	66.39 (11.54)	60.32 (9.60)	55.60 (11.48)

The mean lesser diameter of type I muscle fiber of biceps brachii muscle and vastus lateralis muscle were 63.91±12.28 and 65.65±14.04 μm in males and 60.85±10.68 and 66.39±11.54 μm in females. For type IIA fibers, the values were 62.37±10.47 and 63.90±12.25 μm in males and 58.75±8.38 and 60.32±9.60 μm in females. The corresponding values of type IIB were 59.87±9.78 and 61.67±12.08 μm in males and 55.86± 8.93 and 55.60±11.48 μm in females. (Table 2.)

The student t-test comparing the type I, type IIA and type IIB mean fiber diameters of bicep brachii muscle between males and female showed statistically significant difference ($p<0.001$) with higher mean values for males (Figure 4). For vastus lateralis muscle, the results showed statistically significant difference between males and females with higher mean values of type IIA and type IIB fiber diameter in males ($p<0.001$). In type I, the females's values were higher

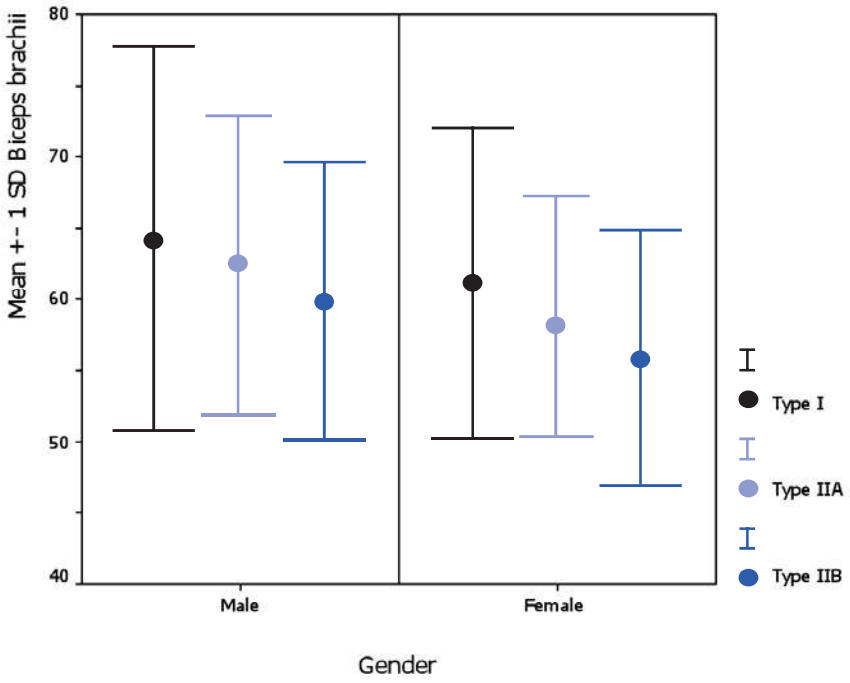

Fig.4 *Mean lesser diameter and SD (μm) of the biceps brachii muscle according to gender*

than males's but the results were not significantly difference (Figure 5)

The mean lesser diameter of type I muscle fiber of biceps brachii and vastus lateralis were 62.09±11.37 and 67.18±14.02 μm in age group < 50 years old and 64.04±12.42 and 64.39±12.42 μm in age group ≥ 50 years old, respectively. For type IIA fibers, the values were 61.36±10.49 and 65.38±11.59 μm in age group < 50 years old and 61.24±9.49 and 60.00±11.05 μm in age group ≥ 50 years old, respectively. The corresponding values of type IIB were 58.57±10.34 and 63.14±12.48 μm in age group < 50 years old and 58.82±8.96 and 56.22±10.807 μm in age group ≥ 50 years old, respectively (Table 3).

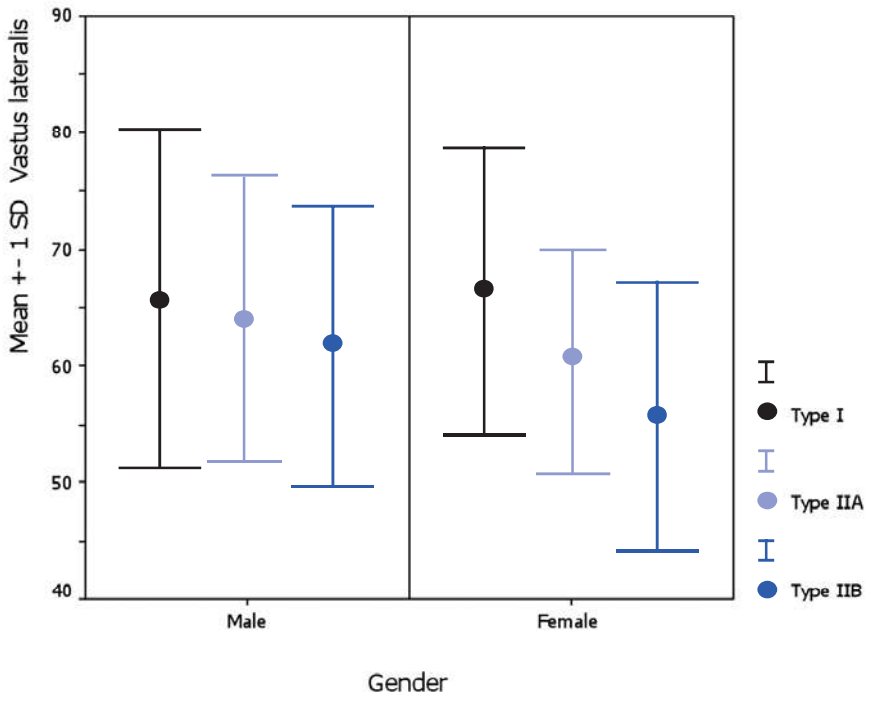

Fig.5 *Mean lesser diameter and SD (μm) of the vastus lateralis muscle according to gender*

Table 3 Mean fiber diameters of biceps brachii and vastus lateralis muscles in age group <50 years (n=10) old and >=50 years old (n=10)

Diameter	Type I Mean (SD) (μm)	Type IIA Mean (SD) (μm)	Type IIB Mean (SD) (μm)
Biceps brachii			
Age < 50 yrs (n=10)	62.09 (11.37)	61.36 (10.49)	58.57 (10.34)
Age ≥ 50 yrs (n=10)	64.04 (12.42)	61.24 (9.49)	58.82 (8.96)
Vastus lateralis			
Age < 50 yrs (n=10)	67.18 (14.02)	65.38 (11.59)	63.14 (12.48)
Age ≥ 50 yrs (n=10)	64.39 (12.42)	60.00 (11.05)	56.22 (10.80)

The comparison of mean fiber diameter between age group less than 50 years old (<50 yrs) and age group 50 years old or more (≥50 yrs), showed that all three fiber types of vastus lateralis muscle in age group less than 50 years old were larger than any fiber in another group with statistically significant (P <0.001). In biceps brachii muscle, there were not significantly different between two age groups (Figure 6 and 7)

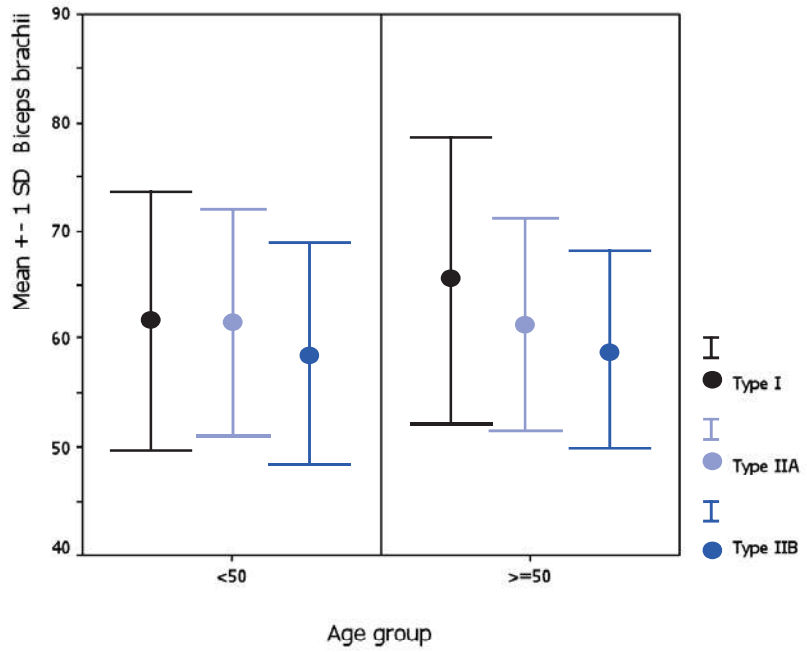

Fig.6 *Mean lesser diameter and SD (μm) of the biceps brachii muscle according to age group*

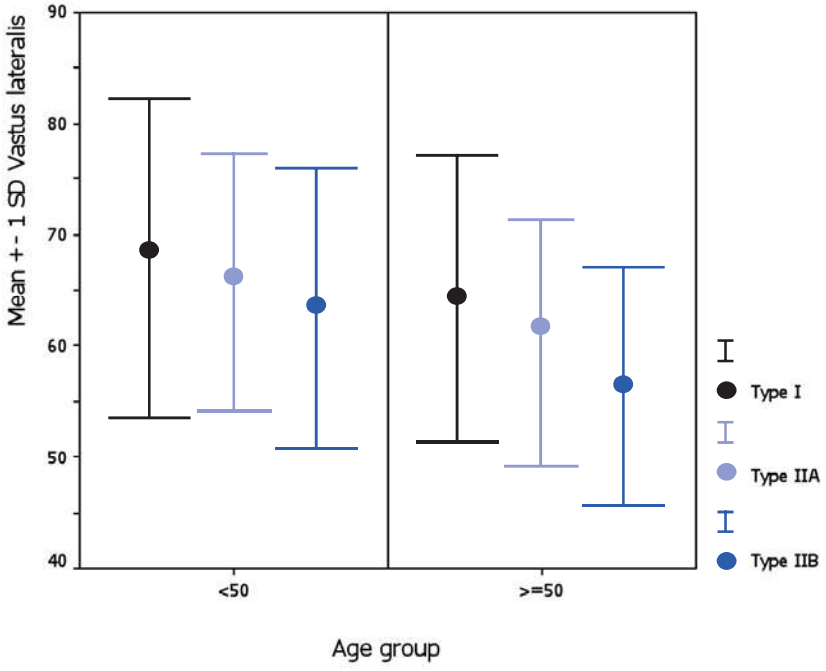

Fig.7 *Mean lesser diameter and SD (μm) of the vastus lateralis muscle according to age group*

Discussion

Postmortem muscle biopsy specimens have some limitations when compared with biopsy specimens obtained from living subjects. The time interval between the death and the removal of muscle specimen is related to the degree of muscle fiber swelling, which are the progressive effects of rigor mortis on the muscle fiber. Differences were also encountered in muscle fiber size during rigor mortis for up to 48 hours after death. However, Shorey and Cleland 9 analyzed morphometric data of deltoid, brachial biceps, vastus lateralis, gastrocnemius and soleus muscles in necropsy specimens of 5 young women taken 18-54 hours after death. For our study, the muscle samples were taken within 36 hours after death.[9]

The fiber type proportion of biceps brachii and vastus lateralis muscle showed predominance of type II fiber and no significance differences between type IIA and type IIB proportions. Type II fiber is a fast twitch fiber, which is a predominance fiber type in muscles such as biceps brachii and vastus lateralis muscles. There were no difference of the fiber type proportion between biceps brachii and vastus lateralis muscle, and between males and females.

Mean lesser fiber diameter of all three fiber type in both biceps brachii and vastus laterlis muscles obtained for both sexes in our study was within the range established by Brooke and Engel[8], 40-80 μm for males and 30-70 μm for females, and 34-71 μm and 34-65 μm for type I fibers, and 36-79 μm and 32-59 μm for type II fibers, in males and females by Mattiello Sverzut et al[10].

In vastus laterlis muscle, mean lesser diameter of the type I, type IIA and type IIB fibers was larger than in biceps brachii muscle, and male was larger than female, supportive that large muscles have a large fiber size and gender had influence on all fiber type.[11,12]

In female, type I fibers were larger than type II fibers, while in males type II fibers were larger than type I, as observed by Brooke and Engel. However, the results of our study showed that, both gender, type I fibers were larger than type II fibers in both biceps brachii and vastus lateralis muscles. A comparison between size of type IIA and type IIB fibers, showed type IIA were larger in males and females. This result was corresponding to other previous studies[1,4,6,8].

In our study, we found that aging had significantly influence on all muscle fiber size of the vastus lateralis muscle in both gender. However, no evidence supported for the biceps brachii muscle.

We concluded that, for non skeletal muscle disease Thai population, there is no gender difference between males and females with regard to the fiber type proportion in the biceps brachii and vastus lateralis muscle. The mean lesser fiber diameter of type I, type IIA and type IIB in non-skeletal muscle disease Thai population was within the interval established from the data published by previously standard reference. However, in our study, male type II fiber is smaller than type I in both biceps brachii and vastus lateralis muscles, which was difference from previously studies. We still had no plausible explanation for this discrepancy. Possibly hypothesis could be differences in sample size, age, and physical activity level. Last, for the vastus lateralis muscle, we found that aging had influence on muscle fiber by decrease fiber size in all three types in males and females.

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