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Non-Invasive estimation of muscle fiber type using ultra Sonography

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Abstract

Introduction: The aim of this study was to estimate the muscle fiber type (i.e., fast/white muscle fibers and slow/red muscle fibers) on the basis of selected muscle architectural parameters (i.e., Pen-nation angle, Muscle thickness and Fascicle Length of Rectus Femoris and Vastus Lateralis) using ultrasound image analysis.

Materials and Methods: In this work of investigation, a total sample comprised of 30 highly trained male athletes of Lakshmbai National Institute of Physical Education Gwalior, Madhya Pradesh was considered as sample for present investigation. Purposive sampling technique was employed for selecting sample, out of which 15 were marathon runners and 15 were 100-meter sprinters. The selected subjects' age ranged between 19 to 25 years. Muscle architectural parameters such as Pen-nation angle, Fascicle length, and Muscle thickness were measured using a B-Mode ultrasonography method (Model-WIPRO GE VOLUSON E 6).

Results: The developed estimate model explains 54.6% to 72.8% of the variation in muscle fiber type. Thus, the logistic regression equation developed was: $-\text{Log } p/(1-p) = 10.955 - 3.278 (\text{VL_FL}) + 1.267 (\text{VL_PA})$.

Conclusion: The Wald statistics obtained from the logistic model revealed that decreasing the vastus lateralis pen nation angle by one unit increases the likelihood of having white muscle fiber by 2.54 times, while increasing the Vastus Lateralis Fascicle Length by one unit increases the likelihood of having white muscle fiber by 0.62 times. In order to determine muscle fiber type on the basis of muscle architectural parameters Vastus lateralis pen-nation angle and fascicle length were the most discriminating variables among selected fiber types.

Keywords: Non-invasive, muscle fiber, ultra sonography, estimation and logistic regression

Introduction

Testing of muscle fiber composition

Early in the twentieth century, the technique of muscle biopsy was developed for the study of muscular dystrophy. It was developed in the 1960s to sample muscles for use in exercise physiology research, specifically to determine the muscle fiber types Slow oxidative (SO) Type 1, Fast oxidative glycolytic (FOG) Type 2a, and Fast glycolytic (FG) Type 2x.

In light of the fact that there is no way to directly determine the fiber type composition of an individual except through the use of an invasive muscle biopsy test (in which a hollow needle is inserted into the muscle and a core sample of muscle fiber is extracted for examination under a microscope), some researchers have attempted to indirectly estimate the fiber type composition within muscle groups of an individual by testing for a relationship between the different properties of fiber type and muscle composition. It has been discovered that there are strong connections between the fraction of FT fibers in the muscle and muscular strength or power in studies conducted with iso-kinetic dynamometers or electrical stimulation (Coyle, E.F., D.L. Costill, 1979; Froese, E.A., 1985; Gerdle, B., M.L. Wretling, 1988; Gregor, R.J., V.R. Edgerton, J.J. Perrine, D.S. Campion, 1979; Suter, E., W. Herzog, J. Sokolosky, J.P. Wiley, 1993) [6, 7, 8, 9, 22,] Accurate determination of muscle fiber composition is invasive and expensive, while in-direct measurement approaches necessitate the use of specialized resources and skills to be effective (Hall *et al.*, 2021) [10]. Hence in this present study researcher tried to estimate muscle fiber type using muscle architectural properties analysis through ultrasonography techniques.

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Muscle Architecture

Gans and De Vries were the first to describe muscle architecture. Muscle architecture refers to the macroscopic arrangement of muscle fibres that controls a muscle's mechanical function (Salimin, 2018) [20]. There are two types of fiber arrangements based on the varied arrangement of skeletal muscle fibers. Muscle fibers are positioned parallel to each other in fusiform muscles (longitudinal fiber arrangement). For example, the bicep femoris and brachioradialis have fiber arrangements that benefit speed, range of motion, and force generation.

Pennate Muscles (diagonal fiber arrangement) - Muscle fibers are arranged in a feather-like pattern, with the tendon replacing the bark. These fiber configurations have a smaller range of motion yet produce more force. Pennate structure can be found in muscles like the rectus femoris and gastrocnemius, which are frequently called upon to create significant forces in order to support or propel the body's weight. A better understanding of the structural arrangement of muscle fibers can be gained by examining the muscular architectural parameters that are listed below.

- Physiological cross-sectional Area (PCSA)
- Pennation angle
- Muscle thickness
- Fascicle Length

Physiological cross-sectional area (PCSA)

Physiological cross-sectional area is the area of a muscle's cross section taken perpendicular to its fibers, which is usually the greatest point on the muscle's cross section.

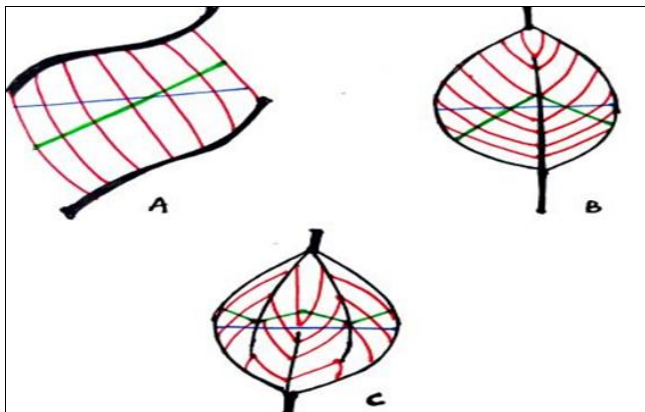


Fig 1: Physiological cross-sectional area (PCSA) and anatomical cross-section area (ACSA).

“The muscle cross-sectional area (blue line in figure 1, also known as anatomical cross-section area, or ACSA) does not accurately represent the number of muscle fibers in the muscle. (Maden-Wilkinson *et al.*, 2021) [25] A better estimate is provided by the total area of the cross-sections perpendicular to the muscle fibers (green lines in figure 1.1). This measure is known as the physiological cross-sectional area (PCSA), and is commonly calculated and defined by the following formula, developed in 1975 by Alexander and Vernon.” (Maganaris C.N., 2000; Narici M.V., Landoni L., 1992; R. McN. Alexander, 1975) [15, 18, 19].

$$PCSA = \frac{\text{Muscle Volume}}{\text{Muscle Fiber Length}}$$

The following is an alternative formula for calculating the PCSA:-

$$PCSA = \frac{\text{Muscle Mass} \times \cos \theta}{\text{Muscle Fiber Length} \times \text{Muscle Density}}$$

Where, $\cos \theta$ stands for the pennation angle/fiber angle

2. Pennation Angle

Pennation angle is defined as the angle formed by a fascicle's orientation with respect to the tendon axis (ref. fig. 2), and it is an important muscle characteristic that has a significant impact on the amount of force contributed by a fascicle to skeletal movement (Salimin, 2018) [20].

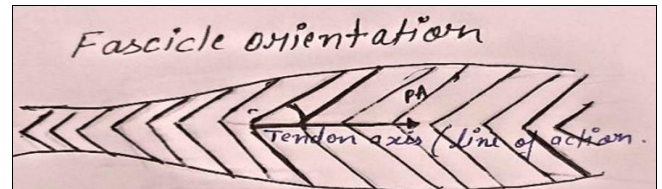


Fig 2: Pennation angle of bipennate muscle.

3. Fascicle Length

Fascicle Length is the length of muscular tissue measured from origin of muscle fibers from proximal end of the bone to the fibers at musculo-tendonous junction forming. (ref. fig. 3) (Kumagai *et al.*, 2000) [13].

Relationship between PCSA, Pennation angle and Length of muscle fiber

The PCSA increases with the pennation angle and with the length of the muscle. In a pennate muscle, PCSA is always greater than ACSA, even when the muscle is not contracted. In a non-pennate muscle, it corresponds to the ACSA gene.

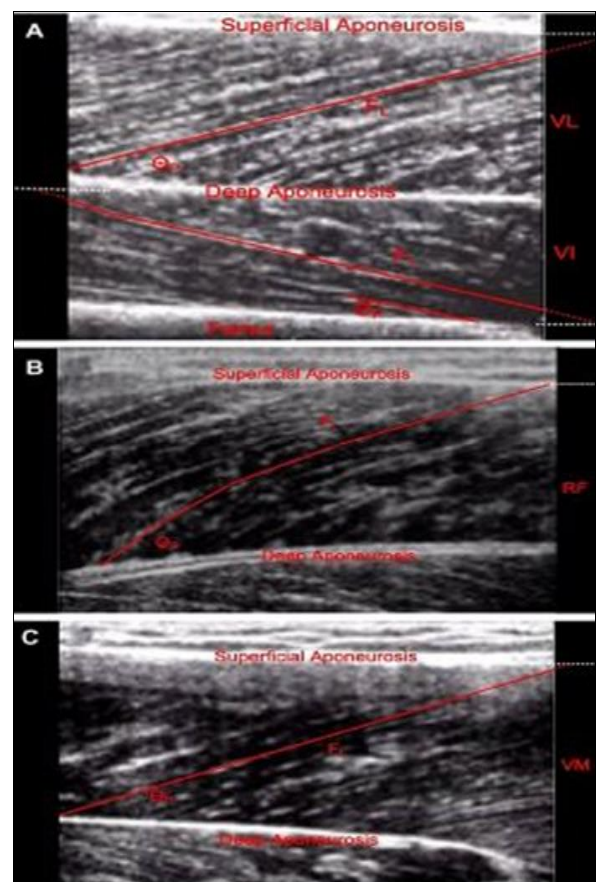


Fig 3: Pennation Angle, Fascicle Length and Muscle Thickness of quadriceps muscle.

4. Muscle Thickness

It is defined as the thickness between two fascias of muscle. (ref. fig. 3) In general thickness considered as the main factor for determining muscle size (Abe *et al.*, 2001) ^[1].

Muscle Architecture Relationship with Fiber Types

Scientific works on muscular architecture are presently trending in this time. Muscle force is directly related to physiological cross-sectional area. Muscle velocity is inversely related to the length of the muscle fibers. Sprinters have longer fascicles than distance runners, and this is reflected in their leg muscle length. Sprinters' leg muscles have a longer fascicle length (vastus lateralis) and a smaller pennation angle than the general population. Greater pennation angle permits a greater quantity of contractile tissue to bind to a given piece of tendon, or aponeurosis, thus increasing the physiological cross-sectional area of a muscle. (Blazevich AJ, Coleman DR, Horne S, 2009; K Albracht, A Arampatzis, 2008; M M Bamman, B R Newcomer, D E Larson-Meyer, R L Weinsier, 2000) ^[4, 12, 14] The increment in pennation angle will causes a cross sectional area of muscle to have more number of fibers. This will therefore boost the muscular ability to produce more force. (Manal K, Roberts DP, 2006) ^[16] discovered pennation angle to be linked with muscle thickness and improvement in strength. However, a increment of pennation angle with constant cross-sectional area has been reported to cause reduction of strength (Ikegawa S, Funato K, Tsunoda N, Kanehisa H, 2008) ^[11]. This condition was assumed to be influenced by the angle of

pull of the fibers that is indirect to the draw of the muscle in total, and thus cause the pull of the muscle in total lowered by the cosine of the pennation angle. Fascicle length is the distance of fascicle from aponeurosis to another aponeurosis. Mathematically, it is a product of fascicle thickness and pennation angle. Fascicle length will be increased with the increment of muscle thickness and decrement of pennation angle. A difference in muscle thickness in the leg muscles (vastus lateralis, gastrocnemius medialis and lateralis) is a significant element in distinguishing sprinters from long distance runners (Salimin, 2018) ^[20].

Materials and Methods

Selection of Subjects

In this work of investigation, a total sample comprised of 30 highly trained male athletes of Lakshmibai National Institute of Physical Education Gwalior, Madhya Pradesh was considered as sample for present investigation. Purposive sampling technique was employed for selecting sample, out of which 15 were marathon runners and 15 were 100-meter sprinters. The selected subjects' age ranged between 19 to 25 years. Required data was collected after taking consent of concerned subject and parents of selected subject.

Selection of Variables

The following variables are selected for study and are presented below along with their criterion measure in the table below: -

Muscle Selected	Muscle Architectural Variable	Criterion Measure	Unit of Measurement
Vastus Lateralis and Rectus Femoris	Muscle fascicle length	Ultra sound Model- WIPRO GE VOLUSON E 6 (B-Mode ultra sound)	Centimeters
	Muscle Pennation Angle		Angle θ
	Muscle Thickness		Centimeter

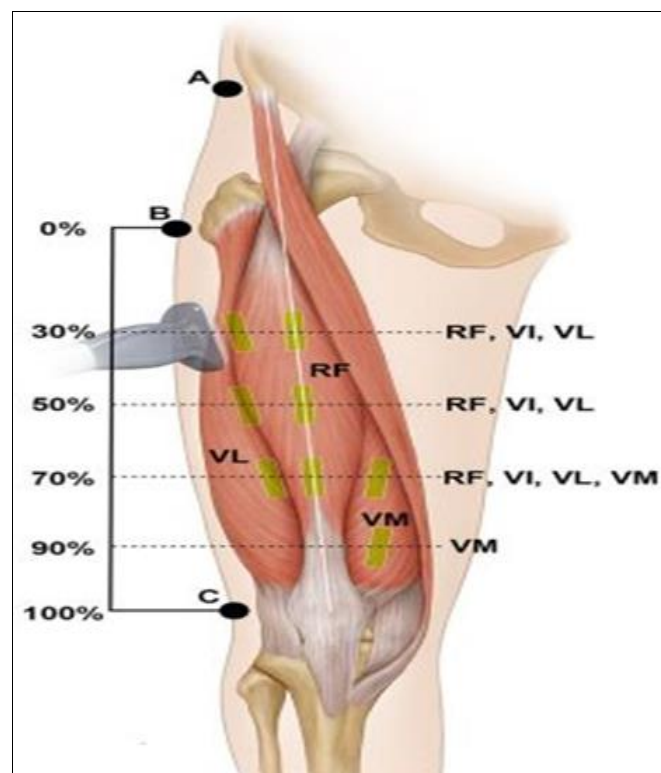


Fig 4: Illustration depicting the measurement sites for the skeletal muscle architectural characteristics. US images were acquired at lengths equivalent to the 30%, 50%, 70%, and 90% levels of the thigh length measured from the greater trochanter to the lateral epicondyle of the femur. The white solid line in A indicates the vertical reference line connecting the anterior superior iliac spine and the center of the patella. The transducer was positioned at the intersection points between the vertical reference line and each measured level (i.e., 30%, 50%, 70%, and 90%). Based on this line, the muscle architectural characteristics of the VL were quantified parallel to the RF and to the VI lines passing through the lateral border of the patella. A, anterior superior iliac spine; B, greater trochanter; C, lateral epicondyle of the femur. RF, rectus femoris; VL vastus lateralis

Determination of muscle architectural parameters.

- **Purpose:** The objective of this test was to determine muscle architectural parameters (Muscle fiber length, Muscle Pennation Angle, Muscle Thickness.) of dominating quadriceps muscles.
- **Equipment:** B-Mode ultra-sonography (Wipro Ge Voluson E), Acoustic gel, 5–10 MHz linear-array transducer (EUP-L53L) and Assistant.
- **Procedure:** Before collecting ultrasound images, participants reported to the laboratory and laid supine for 15 minutes to allow fluid shifts to occur. Following that, non-invasive skeletal muscle ultrasound images of the quadriceps muscles were obtained. To improve spatial resolution, a 12 MHz linear probe scanning head was coated with water soluble transmission gel and positioned on the skin's surface to create acoustic contact without

disturbing the dermal layer to gather the image. All measurements were collected on the dominant leg by the same technician. For each muscle in all individuals, the anatomical position for all ultrasound measurements was standardized.

- **Scoring:** Briefly, pennation angle was measured as the angle of insertion of the muscle fiber fascicles into the deep aponeurosis. Muscle Fascicle length was measured as the length of the fascicular path between the insertions into the superficial and deep aponeurosis, where the fascicular path extended beyond the acquired image the missing portion of the fascicle was estimated by extrapolating linearly the fascicular path and the aponeurosis. The distance between the subcutaneous adipose tissue and intermuscular interface was used to determine muscle thickness.

Table 1: Description of the skeletal muscle characteristics measurement sites. RF, rectus femoris; VL, vastus lateralis.

Muscle	Measurement Site
VL	30%, 50%, and 70% level between the greater trochanter and lateral epicondyle of femur on the line parallel to the RF line passing through the lateral border of patella
RF	30%, 50%, and 70% level between the greater trochanter and lateral epicondyle of femur on the line connecting the anterior superior iliac spine and center of patella

Statistical Analysis

Initially, descriptive statistics were used to analyze the data, which meant that the mean, standard deviation of all the

variables were calculated. Logistic regression techniques was used as the statistical technique for developing estimation model in IBM SPSS 20.0.

Results**Table 2:** Descriptive statistics of Muscle Architectural Variable for selected quadriceps muscles i.e., Rectus Femoris and Vastus Lateralis for different fiber type.

Muscle Architectural Variable	Quadriceps Muscle	Muscle Fiber Type	Mean	Std. Dev.
Pen-nation Angle	Rectus Femoris	White Muscle Fiber	15.1933	1.40024
		Red Muscle Fiber	16.5733	1.74457
	Vastus Lateralis	White Muscle Fiber	15.9933	1.12470
		Red Muscle Fiber	17.9200	1.07251
Muscle Fascicle Length	Rectus Femoris	White Muscle Fiber	9.7353	0.44854
		Red Muscle Fiber	9.6417	0.56051
	Vastus Lateralis	White Muscle Fiber	10.3313	0.52182
		Red Muscle Fiber	9.0867	0.85429
Muscle Thickness	Rectus Femoris	White Muscle Fiber	1.5467	0.32140
		Red Muscle Fiber	1.5173	0.33847
	Vastus Lateralis	White Muscle Fiber	2.5073	0.35320
		Red Muscle Fiber	2.2340	0.35325

The mean and standard deviation of muscle architectural parameter, i.e. pennation angle, Muscle Fascicle Length and Muscle Thickness of selected quadriceps muscles i.e., Rectus Femoris and Vastus Lateralis for different muscle fibre groups are shown Table No. 2. Rectus Femoris white fibres had a pennation angle of $15.19 \pm 1.40^\circ$ while red fibres had a pennation angle of $16.57 \pm 1.74^\circ$. The mean and std. dev. of pennation angle for Vastus Lateralis white fibres was $15.99 \pm 1.12^\circ$ and for red fibres it was $17.92 \pm 1.07^\circ$. Rectus Femoris white fibres had a muscle thickness of 1.54 ± 0.32 cm while red fibres had a muscle thickness of 1.51 ± 0.33 cm. The mean and std. dev. of muscle thickness for Vastus Lateralis white fibres was 2.50 ± 0.35 cm and for red fibres it was 2.23 ± 0.35 cm. Rectus Femoris white fibres had a fascicle length of 9.73 ± 0.44 cm while red fibers had a fascicle length of 9.64 ± 0.56 cm. The mean and std. dev. of fascicle length for Vastus Lateralis white fibers was 10.33 ± 0.52 cm and for red fibers it was 9.08 ± 0.85 cm.

The Hosmer and Lemeshow test was the first output of this logistic regression analysis, as shown in Table No. 3.

Table 3: Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	5.885	8	0.660
2	8.725	8	0.366

The Hosmer and Lemeshow test are used to examine if the generated logistic model is efficient in predicting the occurrence of the dependent variables. This test is used to assess the logistic model's goodness of fit. Hosmer and Lemeshow suggested using chi-square statistics to assess the model's efficiency. The model is judged excellent if the chi-square is negligible. The p value linked with the chi-square is 0.660 and 0.366 for the first and second model, respectively, which is larger than .05. The overall model is statistically significant, $\chi^2(8) = 5.885$ & 8.75 , $p > .05$.

Table No. 4 Classification Table

Observed		Predicted			
		Muscle Fiber Type		Percentage	
		Red Muscle Fiber	White Muscle Fiber	Correct	
Step 1	Muscle Fiber	Red Muscle Fiber	14	1	93.3
	Type	White Muscle Fiber	3	12	80.0
Overall Percentage					86.7
Step 2	Muscle Fiber Type	Red Muscle Fiber	13	2	86.7
		White Muscle Fiber	2	13	86.7
	Overall Percentage				86.7

The classification table compares predicted group membership based on the logistic model to the actual known group membership or percentage accuracy in classification (PAC). The Table No 4 which shows the observed and the predicted values of the dependent variable in both the models. It is clear that the logistic model is effective since the proportion of correctly classified data was 86.7%, which is a respectable number.

Table 5: Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	22.691 ^a	.467	.623
2	17.907 ^a	.546	.728

The explained variation is calculated using the Cox & Snell R Square and the Nagelkerke R Square techniques, as shown in the table above. Pseudo R² values are the name given to these numbers (and will have lower values than in multiple regression).

According to the table no. 5 above, the second model in table no. 4.15 can explain between 54.6% and 72.8% percent of the variation in muscle fiber type based on selected muscle architectural parameters.

Table 6: Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	VL_FL	-3.439	1.428	5.797	1	.016	.032
	Constant	33.935	14.291	5.639	1	.018	5.446E+14
Step 2 ^b	VL_PA	1.267	.672	3.550	1	.060	3.549
	VL_FL	-3.278	1.757	3.481	1	.062	.038
	Constant	10.955	18.502	.351	1	.554	57266.913
a. Variable(s) entered on step 1: VL_FL.							
b. Variable(s) entered on step 2: VL_PA.							

The most significant table displays the value of regression coefficients B (odd ratio), which are required to establish the logistic regression equation for predicting dependent variables from independent variables. The Table No 6 which shows the value of regression coefficients B, Wald statistic, and odd ratio Exp (B) for each variable in both models. The B coefficients are used to develop the logistic regression equation for predicting the dependent variable from the independent variables. These coefficients are in log odds units. Thus the logistic regression equation in the second is:-

$$\text{Log} \frac{p}{1-p} = 10.955 - 3.278 (\text{VL_FL}) + 1.267 (\text{VL_PA})$$

Where, *p* is the probability of having red muscle fiber (reference variable). The dependent variable in the logistic regression is known as logit(*p*) which is equal to:

$$\text{Log} \frac{p}{1-p}$$

The logistic regression equation estimates provided above describe the relationship between the independent and dependent variables, where the dependent variable is on a logit scale. These estimates reveal how much of an increase (or reduction, depending on the sign of the coefficient) in the estimated log chances of "red muscle fiber"=1 would be anticipated by a one-unit rise (or decrease) in the predictor, assuming all other predictors remain constant. (Verma J P, 2013) [23].

In order to make the regression coefficients B more understandable, they are changed into odd ratios equal to Exp (B). Table no. 6's leftmost column has these odds ratios. Only two variables, out of six, can substantially classify muscle fiber types based on identified muscle architectural parameters, as shown in Table 6. The vastus lateralis pennation angle in this model has a higher odd ratio of 3.549, making it the most relevant predictor in predicting muscle fiber type. If the average vastus lateralis pennation angle is greater than white muscle fiber, it may be deduced that the chances of possessing red muscle fiber are enhanced by a factor of 3.549 or vice versa.

With Exp (B) = 3.549, the Wald statistics for VL_PA i.e. Vastus Lateralis Pennation Angle, were revealed to be insignificant (p=0.06). When the odds ratio is greater than one, it means that increasing the independent variable increases the probabilities of the dependent variable odds. That is, if the vastus lateralis pennation angle is increased by one unit, the likelihood of having red muscle fiber increases by 2.54 (3.54-1.00) times or vice versa, assuming all other variables stay unchanged.

With Exp (B) = 0.38, the Wald statistics for VL_FL, i.e. Vastus Lateralis Fascicle Length, were revealed to be insignificant (p=0.062). When the odds ratio is less than one, it means that increasing the independent variable decreases the probabilities of the dependent variable odds. That is, if the Vastus Lateralis Fascicle Length is increased by one unit, the likelihood of having red muscle fiber decreases by 0.62 (0.38-1.00) times or vice versa, assuming all other variables stay unchanged.

According to the findings of logistic regression model, the Vastus Lateralis Pennation Angle and Vastus Lateralis Fascicle Length are the significant contributing variables responsible for identifying muscle fiber based on specified muscle architectural parameters.

Discussion on Findings

Quads white muscle fibers produces more force when compared to Quads red muscle fibers (Clark M, Lucett S, McGill E, 2018; Stacey Penny, 2021) [5, 21]. Quads muscle

fibers lower pennation angle, greater fascicle length and better strength production of white muscle fibers can be explained by using geometric functions also- let's assume an right angle triangle ABC on the surface of Rectus Femoris, Vastus Lateralis, Vastus as shown in figure 6, Where side AC represents the fascicle length, AB represents the Apo-neurosis and BC represents width of the muscle. Angle BAC represents the pennation angle. Considering pennation angle as function of Cosine then,

$$\cos \theta = \frac{AB}{AC}$$

Since the cosine angle decreases the length of AC increases, which clearly establishes the lower pennation angle of muscle allows them to have larger fascicle length. Let's consider AB as \vec{a} , AC as \vec{b} and BC as the resultant vector therefore resultant will be:-

$$R = \sqrt{\vec{A}^2 + \vec{B}^2 + 2 \vec{A} \vec{B} \cos \theta}$$

As, the value of cosine decreases (Cos 17= 0.9563, Cos 16= 0.9612, Cos= 0.9659) its function value increases and overall resultant increases.

Table No. 6 shows the logistic coefficients; Wald test and Odd ratio Exp (E) for each predictor have been shown. Larger

the value of odds ratio more is the predictive value of the independent variables. The first model was constructed by using sixteen parameters namely, RF_PA, RF_FL, RF_MT, VL_PA, VL_FL and VL_MT,. Only two predictors' viz. VL_PA (Wald test=3.55, Exp (E) = 3.549) and VL_FL (Wald test=3.481, Exp (E) = .038) were found to be significant. In other words the one unit decrease in VL_PA, increases the likelihood of having white muscle fiber increases by 2.54 times and one unit increase in VL_FL, increases the likelihood of having white muscle fiber increases by 0.62. Insignificance of Chi square in Hosmer and Lemeshow test in table no. 4.13 showed that the observed model was significant. The developed model is correctly classified as 86.7% original cases (table 4).

Increased fascicle length and its positive relation with fast muscle fiber type dominating activity such as sprinting and lifting was found various studies by (Abe *et al.*, 2001; Baechle TR, 2008; Blazeovich, 2006; K Albracht, A Arampatzis, 2008 and Kumagai *et al.*, 2000) [1, 2, 3, 12, 13]. Lesser pennation angle and its positive relation with fast muscle fiber type dominating activity such as sprinting and lifting was found various studies by (Abe *et al.*, 2001; Kumagai *et al.*, 2000; Salimin, 2018) [1, 13, 20]. In contrasting studies by (Nadzalan *et al.*, 2017; Zaras *et al.*, 2020) [17, 24] greater pennation angle was reported for increased strength performances. But more results were in the favor of this present study.

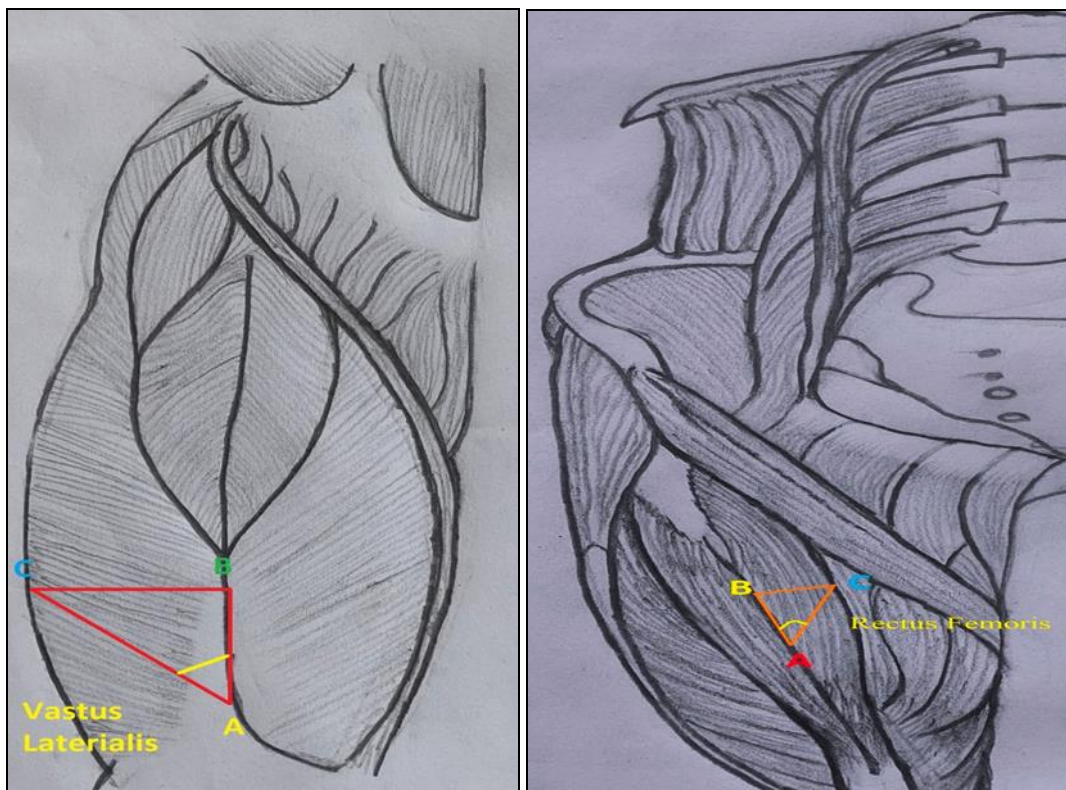


Fig 6: Vastus Lateralis and Rectus Femoris

Conclusion

The present study focused on estimating muscle fiber types on the basis of selected muscle architectural parameters. The muscle architectural parameters were collected using ultrasonography image analysis technique. Based on specified muscle architectural parameters, the developed estimate model explains 54.6% to 72.8% of the variation in muscle fibre type. Thus the logistic regression equation developed was:- $\text{Logp}/(1-p) = 10.955 - 3.278 (\text{VL_FL}) + 1.267$

(VL_PA). The wald statistics obtained from the logistic model revealed that decreasing the vastus lateralis pen nation angle by one unit increases the likelihood of having white muscle fiber by 2.54 (3.54-1.00) times, while increasing the Vastus Lateralis Fascicle Length by one unit increases the likelihood of having white muscle fiber by 0.62 (0.38-1.00) times. In order to determine muscle fiber type on the basis of muscle architectural parameters Vastus lateralis pennation angle and fascicle length were the most discriminating

variables among selected fiber types.

Conflict of Interest

Author declares no conflict of interest.

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