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Effect of age, breed and sex on longissimus dorsi muscle area and subcutaneous fat depth in horses

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ABSTRACT

Muscle mass and strength are important for all horses, but particularly aged horses and horses participating in intense activity, to maintain and improve their ability to perform. The correlation between muscle mass and age, breed, and sex holds implications for use in management in the horse industry, including monitoring changes with age and potential therapeutic treatments and designing specialized training programs based on breed characteristics and sex. The use of ultrasonography for determining muscle characteristics, including area, width, and height, and subcutaneous fat depth provides an inexpensive, noninvasive technique for obtaining these measurements. We hypothesized that muscle area would decrease and subcutaneous fat would increase in adult animals with increasing age, that "easy-keepers" would have greater fat depth than other breeds, and that males would have more muscle than females. Horses from the University of Connecticut herd, ranging in age from 6 months to 21 years, of both sexes and five different breeds, were used to measure longissimus dorsi (LD) muscle area, width, and height, and subcutaneous fat depth. Data was analyzed using the MIXED procedure in SAS. Age, sex, and breed significantly affected LD area and both age and sex had a significant effect on LD height. The area of the LD was greatest in horses 11-15 yr of age ($P < 0.02$) and greater in males than females ($P = 0.0003$). Only age had a significant effect on LD width and fat depth (LD width, $P = 0.0320$; fat depth, $P = 0.0046$). Determining LD muscle characteristics across various ages, breeds, and sexes provides information that may prove useful in the maintenance of muscle mass in aging horses and offers an explanation for the unique abilities of the sexes and of different breeds.

ACKNOWLEDGMENTS

I would like to thank Dr. Sarah Reed, my thesis advisor, for her guidance throughout the research project and the writing of my honors thesis. I would also like to thank my honors advisor, Dr. Kumar Venkitanarayanan, for his advice throughout my four years at UConn and also the Animal Science Department Head, Dr. Steven Zinn, for his assistance in starting the project and his support of my goals. Additionally, I would like to thank fellow undergraduates, Emma LaVigne and Delaney Patterson, for their contributions to gathering and analyzing the data. I would also like to thank Mr. John Bennett and Dr. Jenifer Nadeau for their cooperation and assistance in compiling records of the horses. Lastly, I would like to thank my family, roommates, and friends, for their constant love and support of my dreams. Partial funding for this research was provided by the Life Sciences Honors Thesis Award from the Office of Undergraduate Research (OUR) at the University of Connecticut.

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INTRODUCTION

Horses are widespread throughout the world, used for purposes ranging from pleasure to competition to work. For horses to optimally perform any of these activities, their muscles must be adequate in size, strength, and in a healthy condition. There are numerous factors that affect muscle mass, including: genetics, activity level, age, breed, sex, weight, and environment. Age, breed, and sex are three of the potential influential factors that have yet to be examined in depth in the horse.

The overall number of horses in the United States has been increasing over the years and horses are living longer as a result of improved veterinary care and management practices, meaning there are a greater number of older horses comprising the equine population today (Kilby, 2007). It is vital to understand physiological changes associated with aging in the horse because of the increased number of older horses present in the population. Excluding the racetrack population, 7.8% of equids were 20 years old or older according to the USDA's National Animal Health Monitoring System survey in 1998, a percentage which is likely greater today (Kilby, 2007). Based on this percentage, although likely too low an estimate, there are 780,000 horses that are over the age of 20 out of today's approximately 10 million horses (Kilby, 2007). In 2000, approximately one-half of the Arabian and Morgan populations and greater than one-quarter of the Quarter Horse population were greater than 15 years of age, according to breed registries (Kilby, 2007).

Horses of all ages are engaged in activities such as pleasure and trail riding, dressage, lessons and training, and natural horsemanship. Horse owners are especially concerned with muscle health and care of senior horses to maintain optimal performance and overall health, with

8.7% of horse owners using nutritional supplements for muscle protection during 2012 (American Horse Publications. 2012).

Age and Muscle Mass in the Horse

As with all animals, many physiological changes are associated with the aging horse, such as a decrease in muscle mass and strength (Kim et al., 2005). Sarcopenia, the muscle loss that accompanies aging, can lead to decreases in muscle strength, mobility and function, and injury resistance, causing performance and health issues. Additionally, age has both positive and negative effects on muscle measurements, such as muscle thickness and depth (Valette et al., 1999). Both gluteus muscle depth in the ventral compartment and possibly muscle thickness decrease with increasing age, while cross-sectional area increases with increasing age (Valette et al., 1999). The horses used in Valette et al.'s study, however, were 2-6 years of age, which while not considered aged, does demonstrate the trend as age increases. As age increases, there is atrophy of type II (fast twitch) fibers, an increase in the proportion of type I (slow twitch) fibers, and a decrease in the total number of muscle fibers (D'Angelis et al., 2008; Larsson et al., 1979). There is also a decrease in cross-sectional area of type II fibers and a decreased mitochondrial oxidative capacity in aged individuals (Giresi et al., 2005). Some of the muscle lost with age is replaced by fat and connective tissue, which can impair mobility and function (Larsson et al., 1979). Giresi and Deschenes independently presented evidence that age causes detrimental changes in equine muscle function. Specifically, they recorded decreased maximal shortening velocity and maximal force, as well as fewer fast-twitch motor units in older individuals (Deschenes et al., 2004; Giresi et al., 2005). These changes lead to a decreased performance due to an inability to produce optimal force and continue aerobic metabolism for extended periods.

Some factors aside from advanced age that may influence sarcopenia include the height, weight, genetics, health, activity level, hormones, and sex of the animal (Harris, 1997). Disuse, a sedentary lifestyle, increased age, and female sex promote muscle atrophy. While sarcopenia has been extensively studied in some species, including humans and rats, little research has been performed on the horse. It is important to determine if, and to what extent, horses experience sarcopenia to be able to better alleviate the damaging effects of age, as muscle atrophy may be reversible and preventable.

Breed and Muscle Fiber Type

Different breeds of horses are known for their particular breed characteristics and specialties. For example, Quarter Horses are recognized for their ability to sprint short distances and Thoroughbreds are often used in racing. The numerous breeds of horses have a variety of body types and muscle profiles, which enables them to perform optimally in a variety of specialized activities. While intermediate fibers are the most numerous type in five breeds that have been studied (Belgians, Standardbreds, Thoroughbreds, Quarter Horses, and Welsh ponies), Thoroughbreds have the highest percentage of red fibers and Belgians have the most white fibers (Stull et al., 1980). Red fibers, or oxidative fibers, function using aerobic metabolism and white fibers fatigue more rapidly than the other fiber types because they utilize primarily anaerobic metabolism, while intermediate fibers have properties of both red and white fibers (Stull et al., 1980). Since fiber type is indicative of oxidative capacity, muscle strength, contraction rate, and fatigue resistance, it plays an important role in the special abilities of different breeds. Muscle fiber type differences are due to genetic variations achieved via selective breeding throughout the evolution of the horse. There is a relationship between fiber type and fiber size as well, which influences the muscle mass typical of different breeds. Both fiber type and size are correlated with athletic ability in the horse (Rivero et al., 1993).

Sex as a Major Determinant of Circulating Hormones

Sex plays a major role in the hormones circulating the blood, which contribute to characteristics of muscle throughout the body. Metabolism, including skeletal muscle hypertrophy and atrophy, is regulated via hormones, particularly sex hormones such as estrogens and testosterone (Sipila et al., 2013). Testosterone is an anabolic hormone, meaning it helps synthesize protein and build muscle, leading to increased muscle size (Sipila et al., 2013). It increases muscle size by increasing cross-sectional area in both fiber types and by potentially increasing satellite cell number (Sinha-hikim et al., 2002; Sipila et al., 2013). Satellite cells are quiescent cells on the muscle fiber that are vital for skeletal muscle regeneration (Montarras et al., 2013). These cells are highly active during embryonic development and the perinatal period of muscle growth, but become quiescent a few weeks after birth; in adults, they only become active after injury or degenerative muscle diseases (Montarras et al., 2013). In most species, males are more muscular than females, due, in part, to increased testosterone production in the male body (Bhasin et al., 1997). For example, in male Standardbreds, the muscle thickness of the dorsal and ventral compartments of the gluteus medius is greater than in females, as determined via ultrasound (Valette et al., 1999). Senior care may be improved by adjusting therapies for sex differences.

Importance of Subcutaneous Fat Depth

Subcutaneous fat thickness can be an indicator of metabolism and can assist in determining the optimal feed requirements of individual horses. The amount of subcutaneous fat can show how nutrients are being distributed and utilized by the body. Either an excess or a lack of subcutaneous fat is one method for monitoring an animal's nutritional status. It also can indicate whether any weight gain or loss is due to an increase in muscle or fat (Mirzaei et al., 2009). Ultrasonic subcutaneous fat depth measurements are an accurate predictor of total body fat, an evaluation that has historically been difficult to make (Westervelt et al., 1976). In other species, including cattle, sheep, and swine, fat depth is important in predicting carcass traits to improve yield and quality of meat (Esquivelzeta et al., 2012).

Ultrasonography

Ultrasonography is a powerful tool for examining muscle characteristics in livestock, often times of the longissimus dorsi (LD) muscle. Ultrasonography of the LD muscle has been widely used in the livestock industry to determine muscularity prior to slaughter and to determine the optimal slaughter time for meat animals. The size of the LD is an accurate predictor of meat traits and can be reliably determined via ultrasonography (Kim et al., 2013; Stanford et al., 2001). Muscle fiber characteristics, including fiber number, area, size, and density are related to meat quality traits, including muscle pH, meat color, drip loss, moisture, tenderness, and intramuscular fat content (Kim et al., 2013). The LD muscle is the largest muscle in the equine back and has been repeatedly shown to have great importance in equine mobility (Abe et al., 2012; Ritruechai et al., 2008; Wakeling et al., 2007). Age and adiposity have been associated with decreased muscle mass in animals of other species (D'Angelis et al., 2008; Harris, 1997; Larsson et al., 1979). However, there is currently a limited understanding of how muscle hypertrophies and atrophies with increasing age in the horse.

Ultrasonography also provides a non-invasive method to monitor effects of therapeutic interventions and to track muscle accretion/atrophy and subcutaneous fat depth. Normal muscle in an ultrasound image appears primarily black because muscle has low echo intensity, while bone has high echo intensity and appears as a bright section above a black bone shadow; the ultrasound waves are unable to penetrate bone, so the waves are thus reflected (Pillen, 2010).

Ultrasound imaging is used in lambs to measure subcutaneous fat and the area, width, and depth of the longissimus dorsi muscle (Esquivelzeta et al., 2012; Theriault et al., 2009). Accuracy of measurements taken from ultrasound images has been confirmed in lambs, as well as other livestock species. In sheep, ultrasound images were obtained and the animals were then sacrificed to confirm the ultrasound-based measurements by taking physical measurements (Esquivelzeta et al., 2012). While LD muscle measurements were accurate, there was inconsistency with subcutaneous fat depth repeatability likely due to operator differences or the young age of the animals (Esquivelzeta et al., 2012; Stanford et al., 2001). Two groups of researchers independently examined the reliability and repeatability of ultrasound measurements for equine muscles and their results support additional studies done on other livestock species that indicate the method is both reliable and repeatable (Abe et al., 2012; Lindner et al., 2010). In one study, the thickness of multiple muscles of several Thoroughbred horses was examined, including the extensor carpi radialis, extensor digitorum longus, gluteus medius, longissimus lumborum, semitendinosus, and supraspinatus by multiple examiners for three consecutive days and the measurements were reliable (Lindner et al., 2010). The reliability of ultrasonography was also tested by measurement at four different parts of the longissimus dorsi muscle and measurement of the extensor carpi radialis in Thoroughbred colts, fillies, and mares (Abe et al., 2012). Abe et al. also found the method to be reliable. It is important for the same examiner to take all of the images and for the horse to stand in a relaxed, balanced position so the images captured are reliable and, in turn, the measurements are reliable (Abe et al., 2012; Lindner et al., 2010).

Rationale

While there has been some research on the LD muscle in horses in relation to exercise and in cattle related to age, little is known about the effects of the age, breed, or sex of horses on the LD muscle. We hypothesized that muscle area would decrease and subcutaneous fat would increase in adult animals with increasing age. Horse breeds that are prone to weight gain, such as Morgans, were predicted to have greater fat depth than other breeds. Males were expected to have more muscle than females.

MATERIALS AND METHODS

Animals

Horses (n = 61) from the University of Connecticut herd, ranging in age from 6 months to 21 years, were used. Animals were grouped by breed (Friesian, n = 4; Morgan, n = 25; Quarter Horse, n = 6; Thoroughbred, n = 21; Warmblood, n = 5), age (1-5 yr, n = 18; 6-10 yr, n = 10; 11-15 yr, n = 22; ≥ 16 yr, n = 11), and sex (gelding, n = 23; mare, n = 38). All animal use was approved by the University of Connecticut IACUC.

Ultrasonography

Horses were restrained via halter and lead rope or standing stocks as necessary. An Aloka 500 ultrasound system in B-mode (SSD-500V, Corometrics Medical Systems, Inc., Wallingford, CT) with a 125 mm 3.5 MHz Aloka linear transducer designed for body composition measurements was used to visualize the LD. The area between the 13^{th} and 14^{th} ribs was cleaned using a stiff bristle brush and clipped with grooming clippers. Ultrasound gel was used to facilitate imaging. The probe was placed perpendicular to the body on the clipped section between the 13^{th} and 14^{th} ribs on the left side of the horse. Angle and pressure of the probe were adjusted until a clear image was produced. A minimum of two images were captured for each horse. A single examiner took all images to maintain consistency.

Measurements

Measurements were obtained using AUSKey for Windows 5.10 (Animal Ultrasound Services, Inc., Ithaca, NY) by three independent examiners. Fat depth and LD area, width, and height were measured on each image, and the ratio of height to width was then calculated. Skin was included in the fat depth measurement. Muscle thickness was taken to be the distance from the bottom of the subcutaneous fat to the muscle/bone interface.

Statistics

Data was analyzed using PROC MIXED in SAS with main effects of age, sex, and breed. Differences between means were determined using the LSMEANS statement in SAS. Data is presented as mean ± SEM. *P*-values less than 0.05 are considered significant.

RESULTS

Effects of Age on LD Muscle and Subcutaneous Fat

Age significantly affected LD area, with a significant increase in area between groups until 15 yr of age (Fig 1, $P < 0.02$). The area of horses ≥ 16 yrs of age was significantly smaller than horses 11-15 yr of age (Fig 1, $P = 0.0102$). Age also significantly affected LD height, with a significant increase in height from 1-5 yr to 6-10 yr (Fig 2, $P = 0.0007$) and 6-10 yr to 11-15 yr (Fig 2, $P = 0.0240$), followed by a significant decline from 11-15 yr to ≥ 16 yr. (Fig 2, $P = 0.0012$). There was no significant difference between the 6-10 yr and ≥ 16 yr age groups (Fig 2, $P = 0.6989$). Age also had an impact on LD width. After the significant increase from age 1-5 yr to 6-10 yr (Fig 3, $P = 0.0282$), there was no significant difference in older horses (Fig 3, 6-10 yr v. 11-15 yr, $P = 0.8639$; 11-15 yr v. ≥ 16 yr, $P = 0.4346$). Further, age had no significant effect on the height to width ratio of the LD (Fig 4, $P = 0.1660$). However, subcutaneous fat depth increased significantly from 1-5 yr to 6-10 yr (Fig 5, $P = 0.0021$) and decreased gradually after 6-10 yr, with a significant decrease occurring between 6-10 yr and ≥ 16 yr (Fig 5, $P = 0.0224$).

Effects of Breed on LD Muscle and Subcutaneous Fat

The area of the LD was affected by breed of horse. Friesians, Quarter Horses, and Morgans had the greatest LD muscle area of the breeds studied. The LD muscle area of Friesians was significantly larger than LD muscle area of Warmbloods (Fig 6, $P = 0.0204$) and Thoroughbreds (Fig 6, $P = 0.0045$). The area for Morgans was significantly greater than for Thoroughbreds (Fig 6, $P = 0.0275$). Breed had no significant effect on LD height (Fig 7, $P = 0.1837$), LD width (Fig 8, $P = 0.2778$), LD height to width ratio (Fig 9, $P = 0.9541$), or fat depth (Fig 10, $P = 0.6727$).

Effects of Sex on LD Muscle and Subcutaneous Fat

Sex showed an effect on LD area and height. The LD area was significantly greater in geldings than mares (Fig 11, $P = 0.0003$) and height was significantly larger in geldings than mares (Fig 12, $P = 0.0006$). Sex had no significant effect on width (Fig 13, $P = 0.3268$), height to width ratio (Fig 14, $P = 0.3366$), or fat depth (Fig 15, $P = 0.13$).

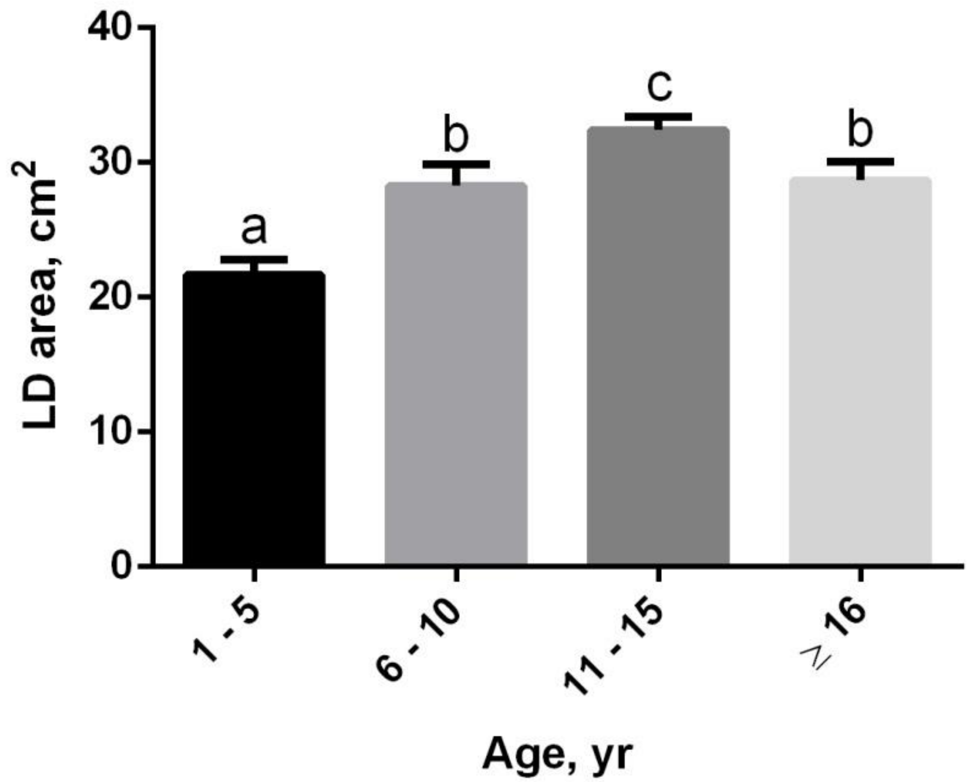

Figure 1: LD area is decreased in aged horses. The area of the LD was measured using ultrasonography. There was a significant increase in area between the 1-5 yr and 6-10 yr groups (*P* = 0.002) and a continued significant increase between 6-10 yr and 11-15 yr (*P* = 0.0183). After 15 yr, the LD muscle area decreased significantly (*P* = 0.0102). There was no significant difference between the 6-10 yr and ≥ 16 yr groups (*P* = 0.8422). Data is presented as mean ± SEM. Columns with different letters indicate significant differences.

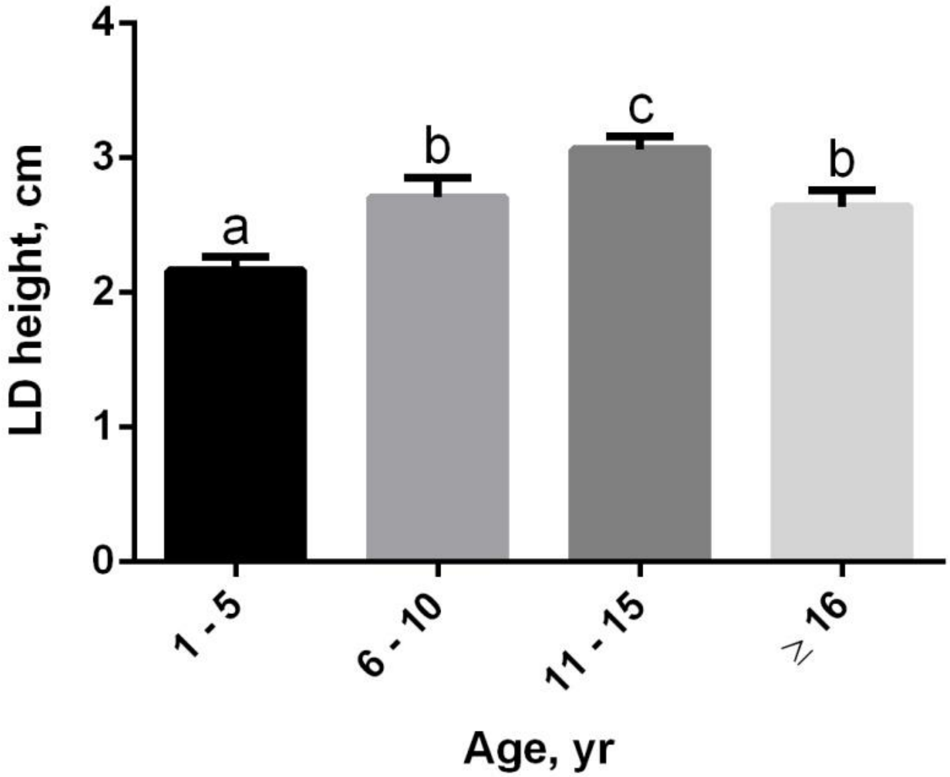

Figure 2: LD height was significantly different between the age groups. The height of the LD was measured in ultrasound images. There was a significant increase in height between the 1-5 yr and 6-10 yr groups (*P* = 0.0007) and a continued significant increase between 6-10 yr and 11-15 yr (*P* = 0.0240). After 15 yr, the LD muscle height decreased significantly (*P* = 0.0012). There was no significant difference between the 6-10 yr and ≥ 16 yr groups (*P* = 0.6989). Data is presented as mean ± SEM. Columns with different letters indicate significant differences.

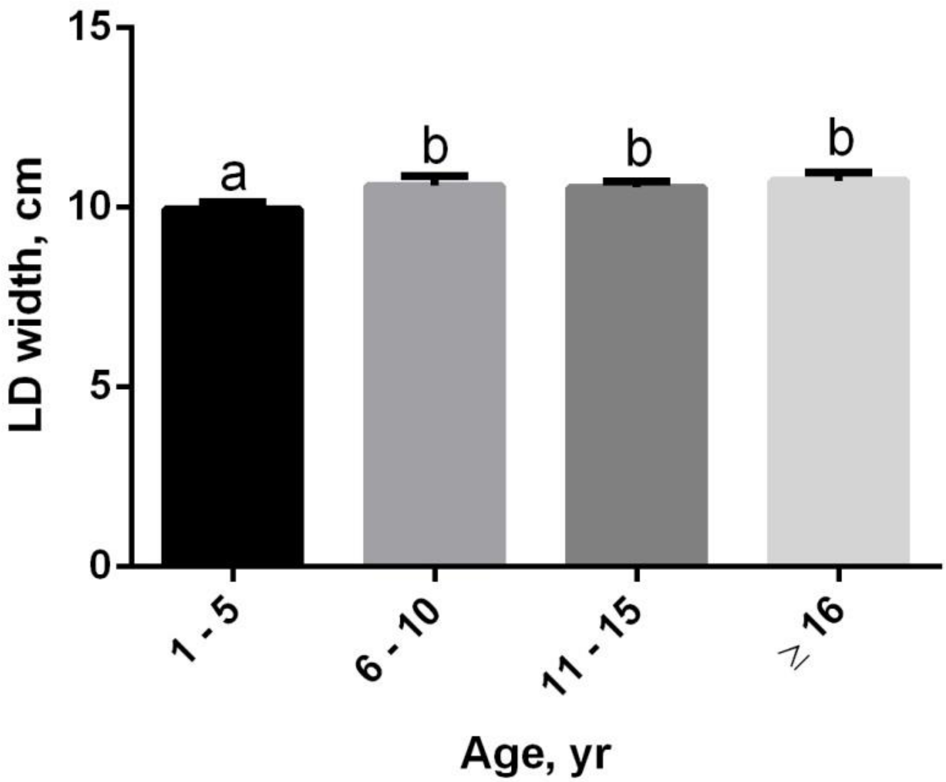

Figure 3: LD width was significantly different between the age groups. The width of the LD was measured in ultrasound images. LD width significantly increased from 1-5 yr to 6-10 yr (*P* = 0.0282) and then remained the same width through ≥ 16 yr. There were no significant changes from 6-10 yr to 11-15 yr (*P* = 0.8639) or from 11-15 yr to ≥ 16 yr (*P* = 0.4346). Data is presented as mean ± SEM. Columns with different letters indicate significant differences.

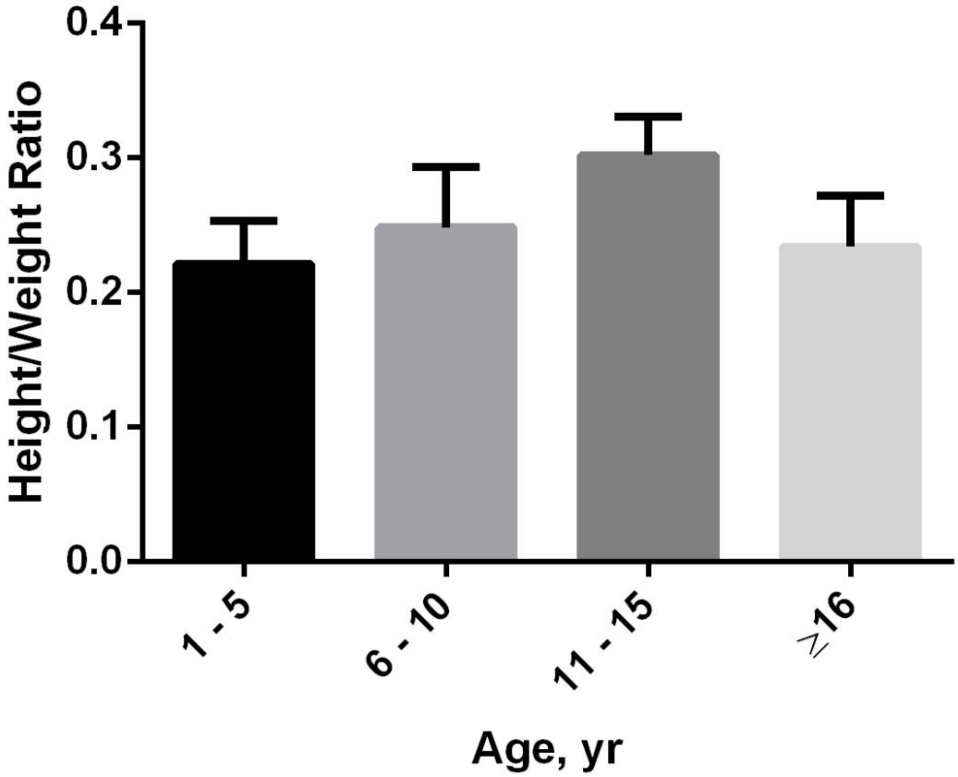

Figure 4: LD height/weight ratio was not significantly different between the age groups. There were no significant differences in the height to width ratio between any of the age groups (P = 0.1660). Data is presented as mean ± SEM.

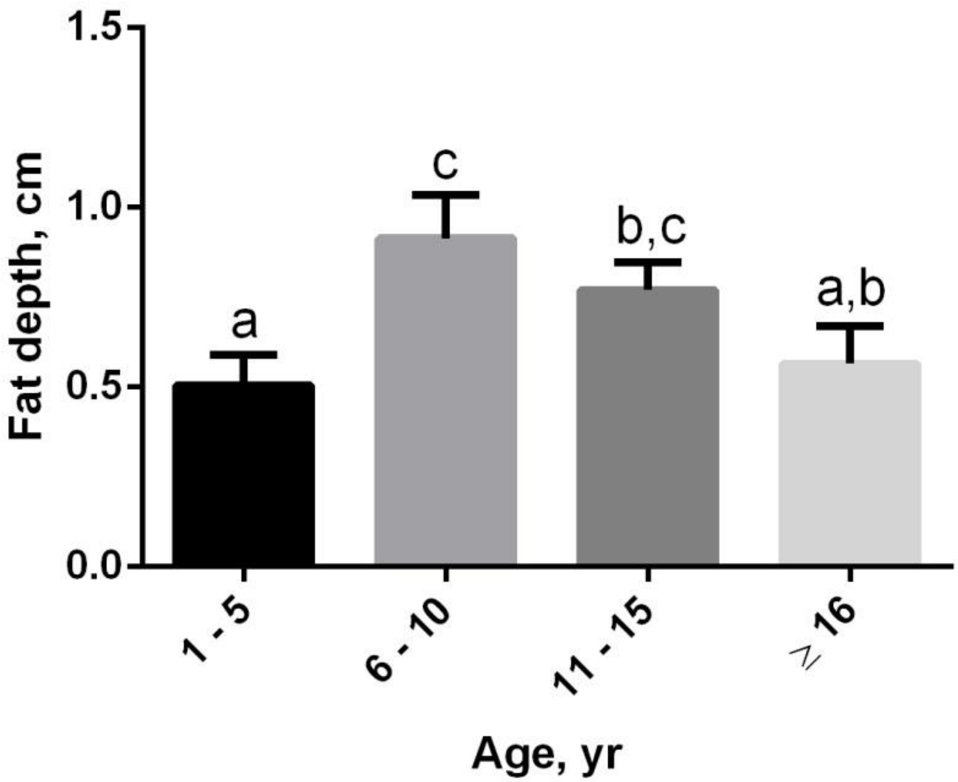

Figure 5: Fat depth was significantly different between the age groups. There was a significant increase in fat depth between the 1-5 yr and 6-10 yr groups (*P* = 0.0021). There was no significant change between 6-10 yr and 11-15 yr (*P* = 0.2763) but was a significant increase between 1-5 yr and 11-15 yr (*P* = 0.0207) and a significant decrease between 6-10 yr and ≥ 16 yr (*P* = 0.0224). There was no significant difference between the 11-15 yr and ≥ 16 yr groups (*P* = 0.0614). There was no significant different between the youngest and oldest age groups (*P* = 0.6547). Data is presented as mean ± SEM. Columns with different letters indicate significant differences.

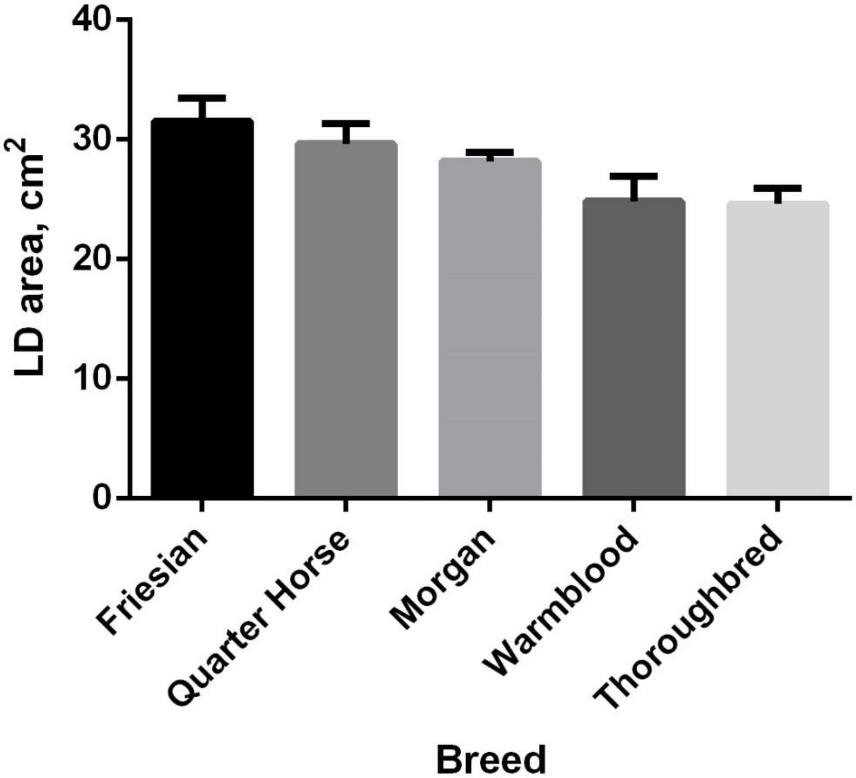

Figure 6: LD area was significantly different between the breeds. The LD muscle area of Friesians was significantly larger than LD muscle area of Warmbloods (*P* = 0.0204) and Thoroughbreds (*P* = 0.0045). The area for Morgans was significantly greater than for Thoroughbreds (*P* = 0.0275). There was no significant difference in the area between Friesians, Quarter Horses, or Morgans (*P* > 0.08), between Quarter Horses, Morgans, or Warmbloods (*P* > 0.08), between Morgans and Warmbloods (*P* = 0.1380), or between Warmbloods and Thoroughbreds (*P* = 0.9251). Data is presented as mean ± SEM.

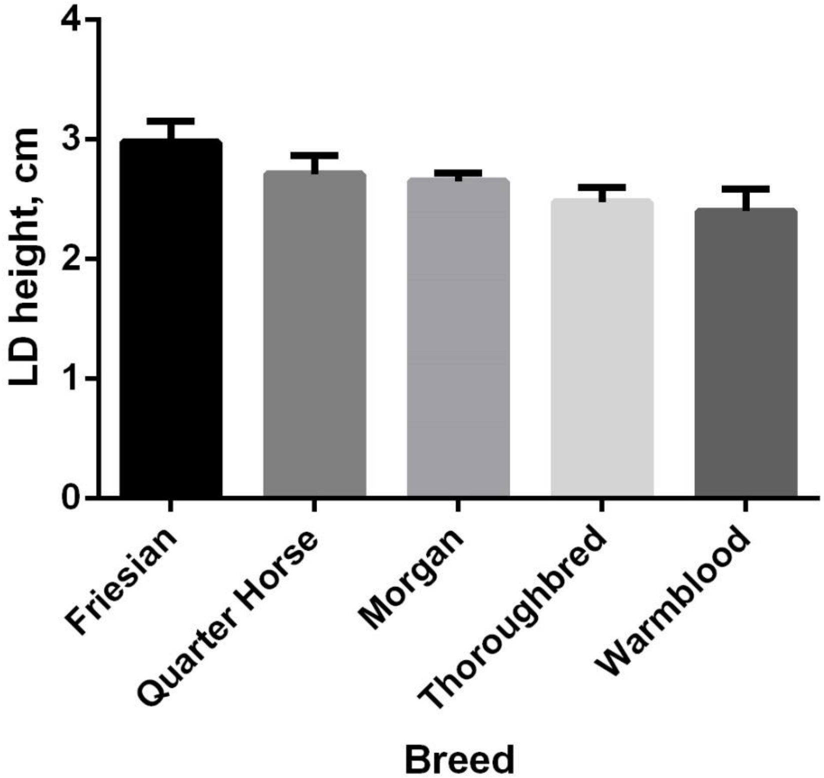

Figure 7: LD height was not significantly different between breeds. There were no significant differences seen in the LD height between any of the breeds ($P = 0.1837$). Data is presented as mean ± SEM.

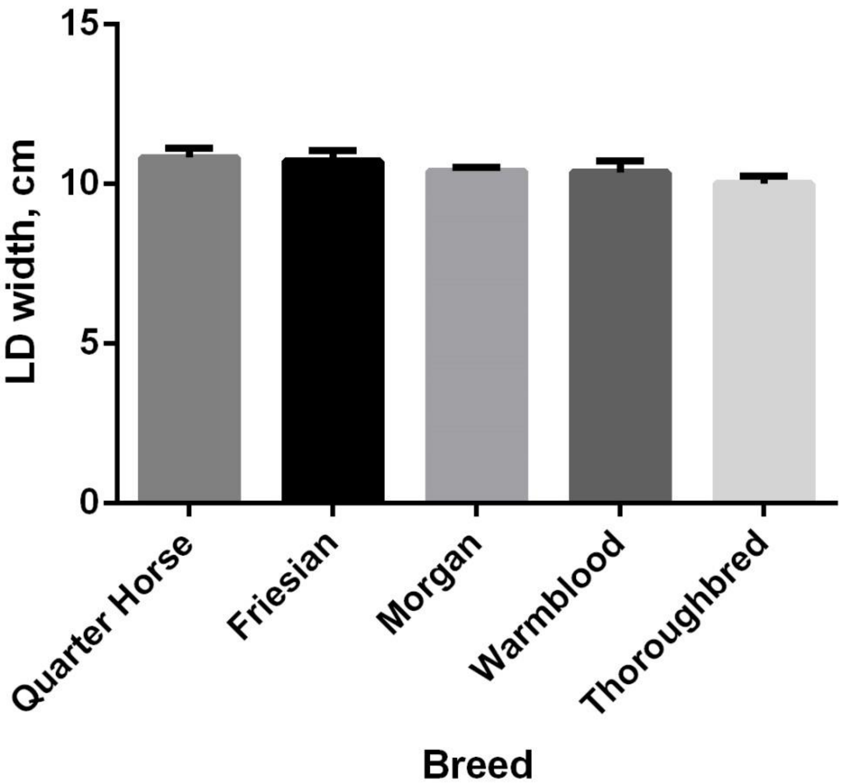

Figure 8: LD width was not significantly different between breeds. There were no significant differences seen in the LD width between any of the breeds (P = 0.2778). Data is presented as mean ± SEM.

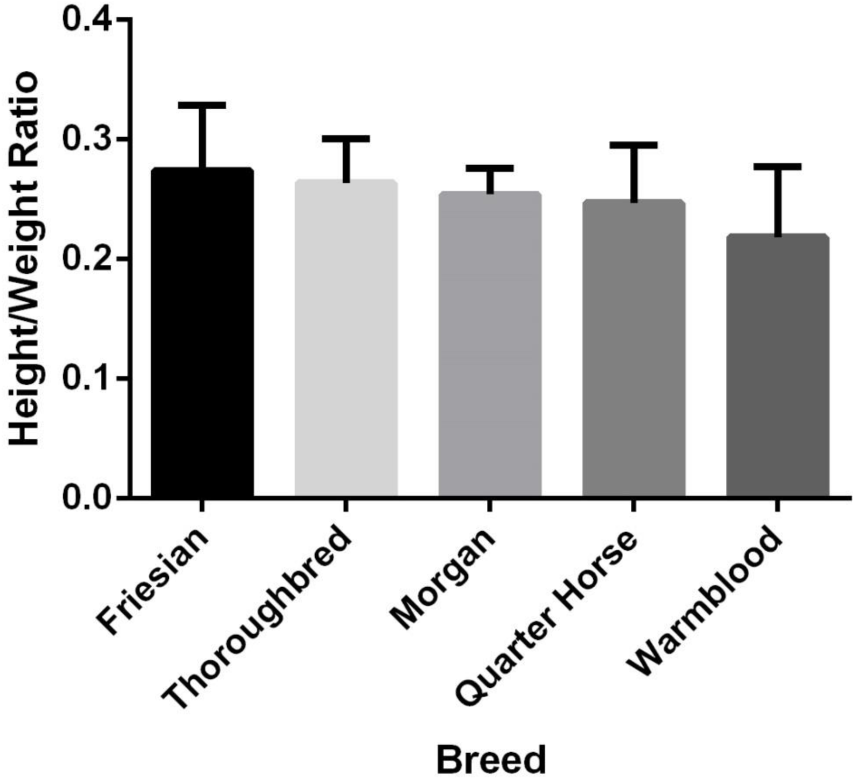

Figure 9: LD height/weight ratio was not significantly different between breeds. There were no significant differences seen in the height to width ratio between any of the breeds (P = 0.9541). Data is presented as mean ± SEM.

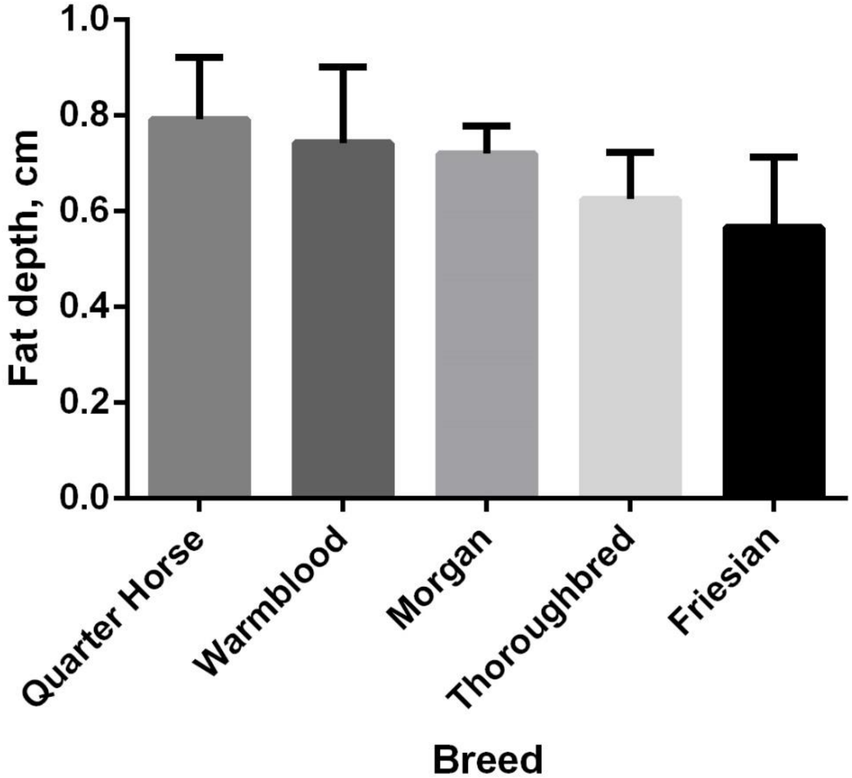

Figure 10: Fat depth was not significantly different between breeds. There were no significant differences seen in the fat depth between any of the breeds (*P* = 0.6727). Data is presented as mean ± SEM.

Figure 11: LD area was significantly different between the sexes. The LD area was significantly greater in geldings than mares. Data is presented as mean ± SEM. * $P = 0.0003$

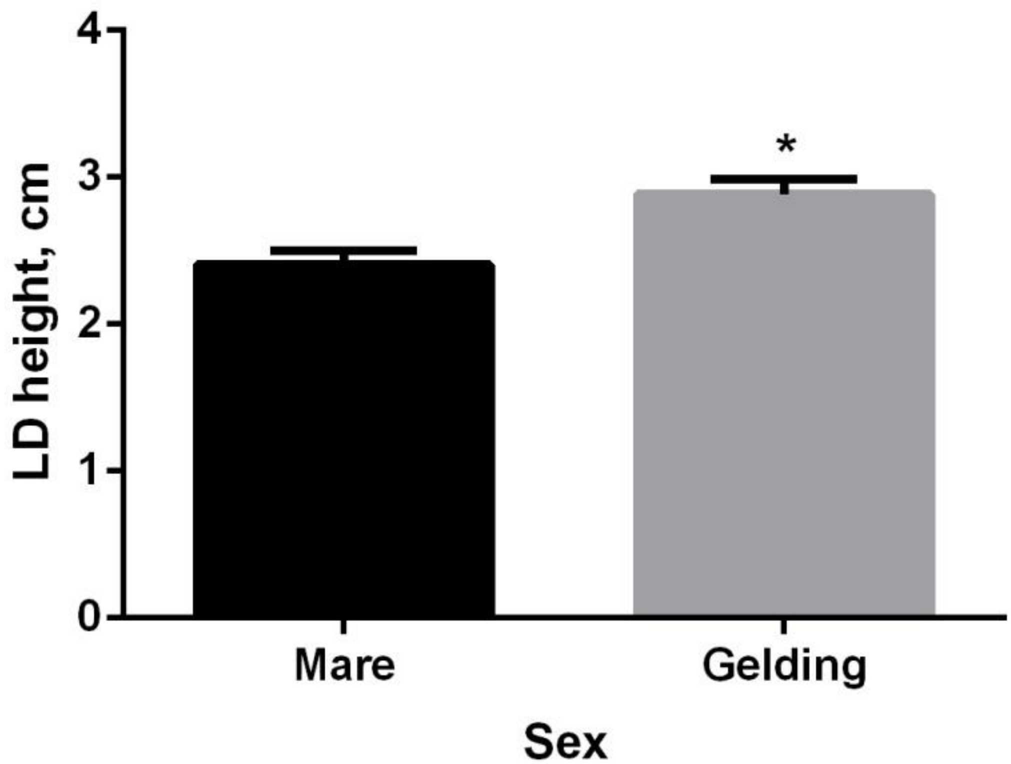

Figure 12: LD height was significantly different between sexes. The LD height was significantly larger in geldings than mares. Data is presented as mean ± SEM. * P = 0.0006

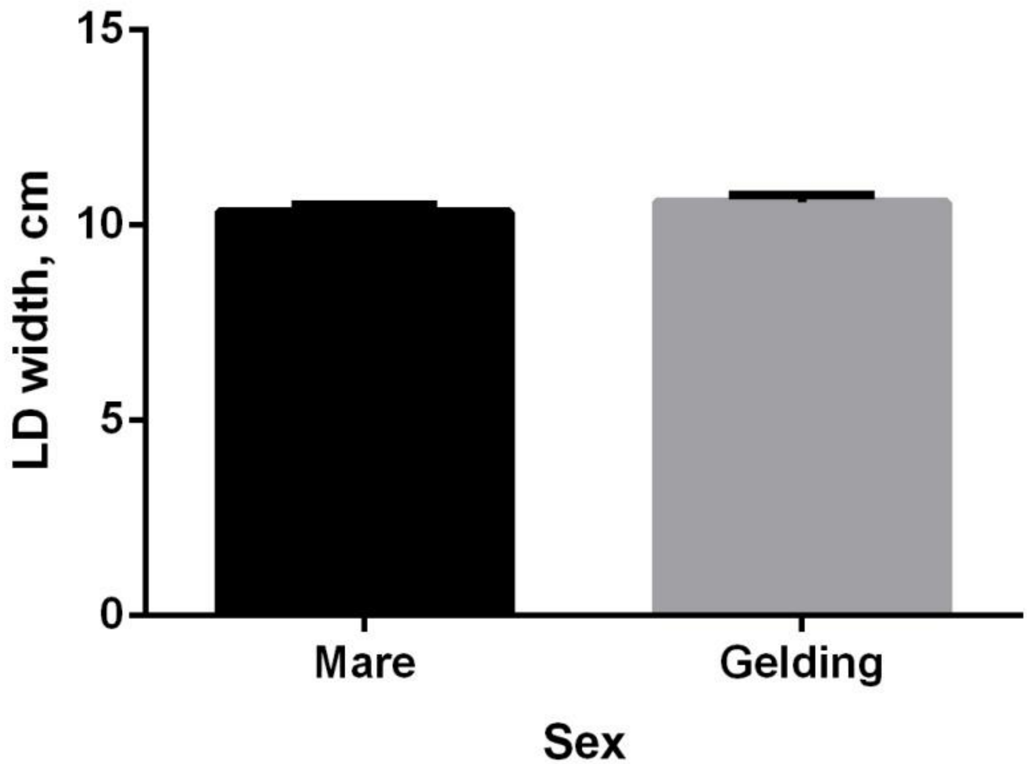

Figure 13: LD width was not significantly different between sexes. There were no significant differences seen in the LD width between the sexes (*P* = 0.3268). Data is presented as mean ± SEM.

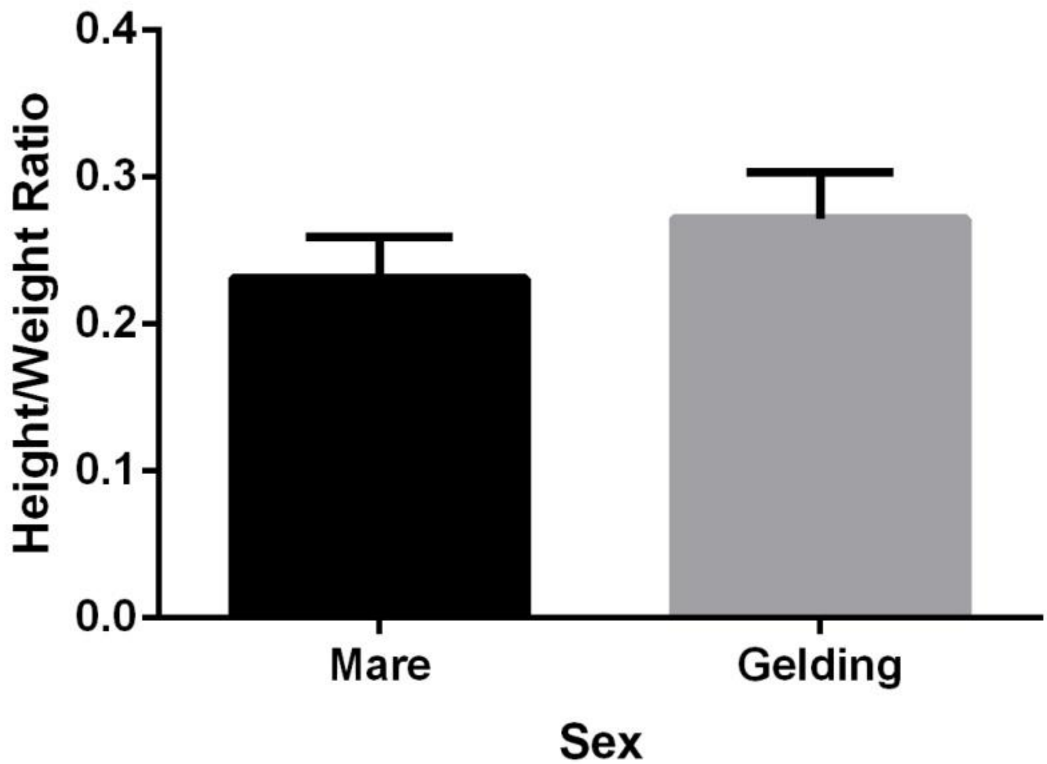

Figure 14: LD height/weight ratio was not significantly different between the age groups. There were no significant differences seen in the height to width ratio between the sexes (P = 0.3366). Data is presented as mean ± SEM.

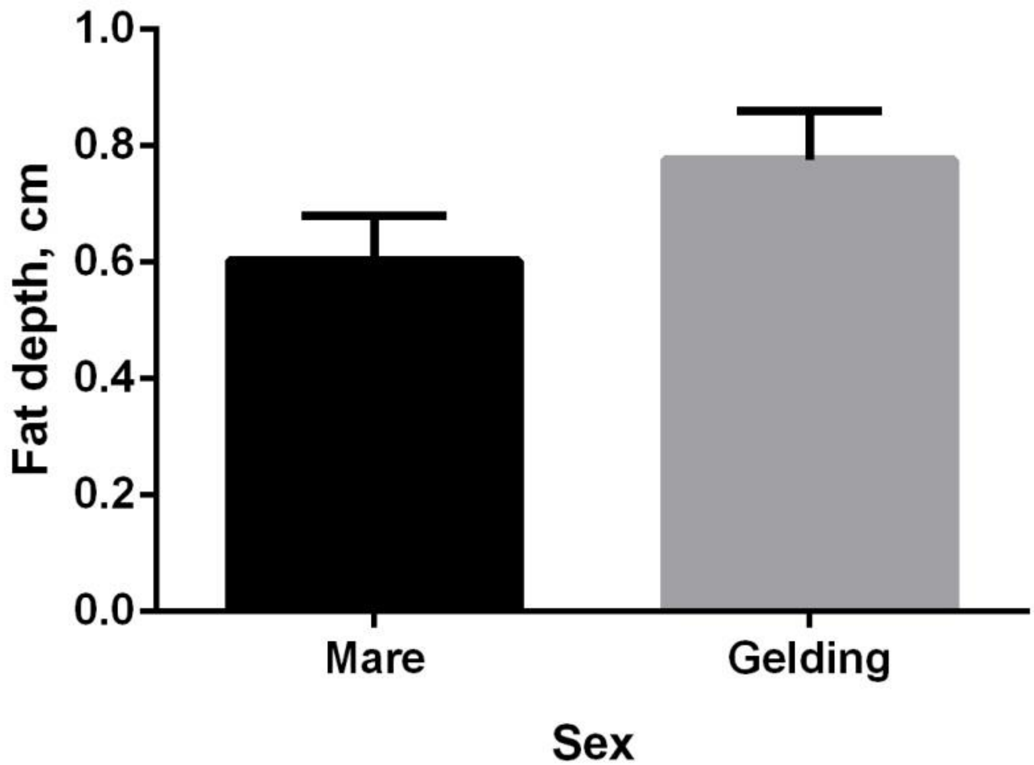

Figure 15: Fat depth was not significantly different between sexes. There were no significant differences seen in the fat depth between the sexes (P = 0.1300). Data is presented as mean ± SEM.

DISCUSSION

As expected, age had a significant effect on most of the measurements taken – LD area, height, width, and subcutaneous fat depth. As seen in other species, muscle mass is highly dependent on age. The increase through 11-15 yr followed by a decrease at ≥ 16 yr observed between age and LD height is the same correlation that occurred between age and area, indicating that muscle characteristics increase with increasing age until reaching a peak between 11-15 yrs. For both area and height, the measurements for the 6-10 yr group were not significantly different from that of the ≥ 16 yr. Not surprisingly, subcutaneous fat depth showed a similar pattern to muscle area and height, but peaked at 6-10 yrs rather than 11-15 yrs. This implies that weight loss begins prior to muscle atrophy. Although we hypothesized that subcutaneous fat depth would increase with age while muscle area decreased, the observation that fat depth also decreased after reaching a peak is understandable, because weight loss is often associated with aging, as well. While LD width was also influenced by age, the effect was only significant between 1-5 yrs and 6-10 yrs, signifying that the width of the muscle grows early on, but maintains a constant width after reaching maturity that is maintained throughout the senior years. Neither age, breed, nor sex had a significant effect on the height to width ratio, although some parameters did have an effect on height or width separately.

Overall, breed and sex had effects on fewer parameters than age, but all three influenced LD area. Breed only affected LD area, with Friesians, Quarter Horses, and Morgans having the largest area and Warmbloods and Thoroughbreds the smallest. This suggests that the powerful function of muscle in Warmbloods and Thoroughbreds is optimized for sport and for racing, respectively, by means other than increased muscle area. Perhaps their ability is due to the fiber type distribution, individual fiber area, innervation, or metabolism. Differences observed between breeds are based on body type differences resulting from genetic variations. For example, some horse breeds, including Morgans, are considered "easy-keepers," as it is relatively easy to maintain their body weight above a certain level. On the other hand, other breeds, such as Thoroughbreds, require much more feed to maintain body weight. This is also due, in part, to the activity level of various breeds. There was surprisingly no significant difference in subcutaneous fat depth between breeds.

Sex was only significantly influential on LD area and height. As expected, geldings had greater muscle area and height than mares. This is likely due to increased testosterone levels in males, because testosterone is an anabolic hormone that causes increased muscle mass.

Future studies should utilize the ultrasonic methods outlined in our current experiment to non-invasively measure the effects of therapies to improve muscle function or to reverse muscle atrophy. Our data may serve as a basis for comparing what parameters affect muscle characteristics in future studies in horses. It would be beneficial to examine the correlation between age, breed, and sex on the muscle characteristics and subcutaneous fat depth in a larger group of horses with greater numbers in each category.

While LD height and width, height to width ratio, and subcutaneous fat depth may be more subject to individual variation or other factors, the overall area is affected by age, breed, and sex. Determining the changes in the LD muscle associated with age and the differences based on breed or sex can provide information that may assist in maintaining muscle mass as horses age and may provide insight into the abilities of the different breeds and sexes. Since there is a potential link between athletic ability and the LD muscle in horses, it would be interesting to further explore the differences between breeds to determine, particularly in Thoroughbreds, what accounts for their superior racing ability. The results are applicable to multiple areas in the horse industry, as many of the horse breeds utilized in this study are included in the top nine horse breeds in the U.S. (Kilby, 2007). Another important implication of this study is the use of ultrasonography, which may provide a non-invasive method to monitor effects of therapeutic interventions and to track muscle accretion/atrophy.

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