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Fiber type and metabolic characteristics of skeletal muscle in 16 breeds of domestic dogs

Kathryn M. van Boom[1] | **Johan P. Schoeman**[2] | **Johan C. A. Steyl**[3] | **Tertius A. Kohn**[1,3,4]

[1]Division of Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town, Cape Town, South Africa

[2]Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

[3]Department of Paraclinical Sciences and Centre for Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

[4]Department of Medical Bioscience, Faculty of Natural Sciences, University of the Western Cape, Cape Town, South Africa

Correspondence
Tertius A. Kohn, Department of Medical Bioscience, Faculty of Natural Sciences, University of the Western Cape, South Africa.
Email: tkohn@uwc.ac.za

Abstract

The domestic dog (*Canis lupus familiaris*) species comprises hundreds of breeds, each differing in physical characteristics, behavior, strength, and running capability. Very little is known about the skeletal muscle composition and metabolism between the different breeds, which may explain disease susceptibility. Muscle samples from the *triceps brachii* (TB) and *vastus lateralis* (VL) were collected post mortem from 35 adult dogs, encompassing 16 breeds of varying ages and sex. Samples were analyzed for fiber type composition, fiber size, oxidative, and glycolytic metabolic capacity (citrate synthase [CS], 3-hydroxyacetyl-coA dehydrogenase [3HAD], creatine kinase [CK], and lactate dehydrogenase [LDH] enzyme activities). There was no significant difference between the TB and VL in any of the measurements. However, there were large intra species variation, with some variables confirming the physical attributes of a specific breed. Collectively, type IIA was the predominant fiber type followed by type I and type IIX. The cross-sectional areas (CSA) of the fibers were all smaller when compared to humans and similar to other wild animals. There was no difference in the CSA between the fiber types and muscle groups. Metabolically, the muscle of the dog displayed high oxidative capacity with high activities for CS and 3HAD. Lower CK and higher LDH activities than humans indicate a lower and higher flux through the high energy phosphate and glycolytic pathways, respectively. The high variability found across the different breeds may be attributed to genetics, function or lifestyle which have largely been driven through human intervention. This data may provide a foundation for future research into the role of these parameters in disease susceptibility, such as insulin resistance and diabetes, across breeds.

KEYWORDS
canine, domestic animal, metabolism, myosin heavy chain

1 | INTRODUCTION

Dogs (*Canis lupus familiaris*) were the first species of animal to become domesticated. The exact dates of domestication are, however, unknown with evidence ranging from 100,000 to 14,000 years ago (Bradshaw, 2006). The advent of kennel clubs in the 19th century led to an increase in selective breeding by humans to align with specific *breed standards*—which clustered dogs with similar traits, lineage, appearance or function (FCI, 2019; KC, 2019). This selective and restrictive breeding has led to over 350 breeds with major phenotypic diversity (from a Chihuahua to a Great Dane) and genetic variation between breeds, but with limited genetic variation within a breed (Ostrander et al., 2017). As a result, the genetic similarity between breeds often correlates with morphological similarities and shared geographical origin as was shown by Parker et al. (2004). Therefore, the present study has classified breeds according to FCI *breed standards* (Braga et al., 2016; FCI, 2019) (Figure 1).

Skeletal muscle is a heterogeneous tissue consisting of a variety of muscle fiber types with varying functional characteristics that may differ between species (Acevedo & Rivero, 2006). The contractile properties of an individual muscle fiber are dependent on the myosin heavy chain (MHC) isoform that it expresses—namely, MHC I, IIA, and IIX, giving rise to Type I, Type IIA, and Type IIX fibers, respectively (Bottinelli, 2001; Kohn et al., 2011). Type I fibers have slow contraction speeds and primarily rely on oxidative metabolism to supply the required ATP. Moreover, Type I fibers are also highly resistant to fatigue, with small cross-sectional areas (CSA), and generally have a higher number of mitochondria and glucose transporter 4 (GLUT4) content compared to Type IIA and IIX fibers (Daugaard et al., 2000; Kohn et al., 2011; Lefaucheur, 2010). In contrast, glycolytic metabolism is the main source of energy in Type IIX fibers which have large CSA and powerful contractions, but fatigue quickly (Kohn et al., 2011; Lefaucheur, 2010). Type IIA fibers, in turn, exhibit a mixture of Type I and Type IIX characteristics. Type IIB fibers have very fast contraction speeds and are generally only expressed in smaller animals or specialized muscles in humans (Toniolo et al., 2007). It is not uncommon for a fiber to express more than one form of MHC simultaneously, termed hybrid fibers (Hämäläinen & Pette, 1995). These fibers bridge the gap between "pure" fibers and are believed to fine tune the efficiency of the muscle (Acevedo & Rivero, 2006; Hämäläinen & Pette, 1995).

From the above, it is clear that the different muscle fiber types have varying oxidative and glycolytic capabilities, each complementing the needs of the contractile properties of the fiber. Due to their high oxidative capacity, Type I fibers make use of substrates from blood such as glucose, free fatty acids, and O_2 to yield energy (Frayn, 2009; Frontera & Ochala, 2015). In contrast, Type IIX fibers rely on anaerobic glycolysis and make use of glucose-6-phosphate produced from glycogen breakdown within the muscle. However, more oxidative Type IIA and IIX fibers have been found in antelope and rodent species compared to humans (Gouspillou et al., 2014; Kohn, 2014). In human muscle, Type I and IIA are the predominant fiber types, with relatively low numbers of Type IIX fibers compared to animals (Curry et al., 2012; Frayn, 2009; Kohn et al., 2011).
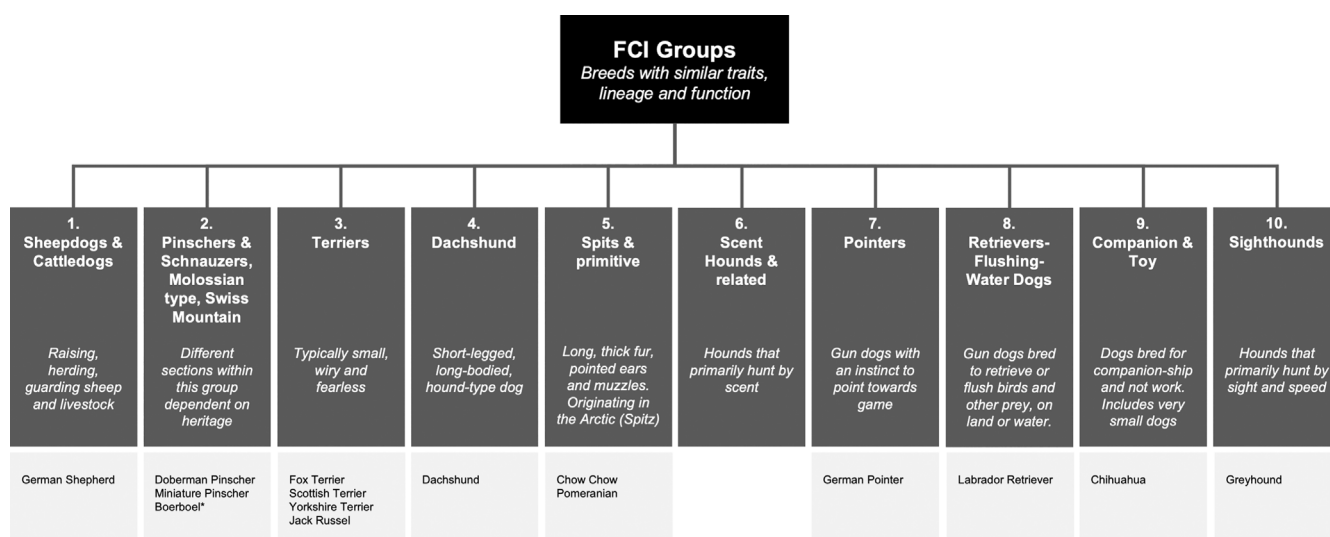

FIGURE 1 Grouping of different dog breeds according to the Fédération Cynologique Internationale (FCI) breed standard. Light gray box indicate dog breeds included in the study sample. *The Boerboel is a South African breed that is not recognized by the FCI, however it is closely related to the Bulldog and Mastiff which fall in Group 2. Mixed Breed dogs are excluded from this figure

An important characteristic of skeletal muscle is its ability to adapt to physiological stress (Schiaffino & Reggiani, 2011). In this regard, exercise training has been shown to cause changes in fiber type composition in humans, with endurance training leading to an increase in Type I fibers, while resistance and sprint training leads to a shift to Type IIA fibers (Andersen & Henriksson, 1977; Trappe et al., 2006). Due to genetics, there is also variability in fiber type composition between species that echo their physical ability. For example, the lion has significantly more Type IIX fibers and higher glycolytic capacity in the *vastus lateralis* (VL) compared to humans, while antelope species, such as blesbok and mountain reedbuck, have higher proportions of Type IIX and IIA fibers that exhibit a higher oxidative capacity (Kohn, 2014; Kohn et al., 2011). The consensus, therefore, is that animals (including humans) that have speed and power, have greater proportions of Type II fibers, whereas those with superior endurance capacity (especially humans) will harbor a greater number of type I fibers. This same pattern may be present in domestic dogs, because they all exhibit different physical attributes—some have been bred for hunting, while others have been bred for companionship.

Over the past 50 years, a handful of studies have been published that investigated the muscle composition and metabolic characteristics of dogs, each with varying research questions and results (Acevedo & Rivero, 2006; Braga et al., 2016; Braund et al., 1982; Gunn, 1978; Guy & Snow, 1981; Kuzon et al., 1989; Latorre et al., 1993; McKenzie et al., 2005; Snow et al., 1982; Strbenc et al., 2004; Toniolo et al., 2007). Overall, the muscle of dogs seem to harbor a prevalence of Type I and IIA fibers with a higher oxidative capacity compared to glycolytic capacity. However, many of these studies had very small sample sizes and did not always differentiate between breeds. Since muscle fiber type may play an important role in various diseases and conditions, such as insulin resistance and Type 2 diabetes, it would be valuable to investigate muscle characteristics in a wider variety of dog breeds (Duan et al., 2017).

Therefore, the aim of this study was to investigate the skeletal muscle fiber composition and metabolic profile in the VL and *triceps brachii* (TB) muscles of 16 domestic dog breeds (including mixed breed).

2 | MATERIALS AND METHODS

2.1 | Study animals

The study sample included 35 adult dogs of various breeds, age, sex, and weight (Table 1). These dogs were euthanised for reasons not disclosed to the researchers. Sampling was opportunistic and collection took place at the Onderstepoort Veterinary Academic Hospital, University of Pretoria and a veterinary hospital in Cape Town, with consent from the owners.

2.2 | Ethics

Ethical approval for this study was obtained from the Faculty of Health Science Animal Ethics Committee, University of Cape Town (Reference number: 017/023) and the Faculty of Veterinary Science Animal Ethics Committee, University of Pretoria (Reference number: v026-18). A Department of Agriculture, Forestry and Fisheries Section 20 research permit was also obtained (Reference number: 12/11/1/7) to ensure compliance with the spread of animal diseases.

2.3 | Tissue collection

Muscle samples were collected post mortem according to the procedure described by Kohn et al. (2011). No dogs were euthanised solely for the purpose of this study. A sample of approximately 100–200 mg were obtained from the mid VL and lateral head of the TB and temporarily stored cold wrapped in gauze wetted with saline at 4°C. After collection, the muscle samples were visually inspected to determine the fiber orientation and samples were cut into smaller longitudinal pieces (ranging from 3 to 5 mm in diameter), rapidly frozen in liquid nitrogen and stored at −87°C until analyses.

2.4 | Section preparation, fiber typing, and fiber CSA

Frozen muscle samples were mounted directly onto the cryostat chuck using Tissue-Tek OCT compound to allow transverse sectioning at −20°C. Serial cross-sections of 10 μm of each muscle were prepared using a cryostat (Leica CM1100; Leica Biosystems) and mounted onto glass slides. The fiber type of each VL and TB muscle sample was determined using immunohistochemistry as described by Dada et al. (2018) with modifications. Briefly, sections were fixed in acetone before blocking nonspecific binding sites with 5% donkey serum made up in 0.15 M phosphate-buffered saline (PBS) pH 7.4 for 1 hr at room temperature. The sections were then incubated overnight at 4°C with primary antibodies (all purchased from Developmental Studies Hybridoma Bank, Iowa City, IA) at a dilution of 1:50 in PBS against the MHC corresponding to Type I (BA-D5; monoclonal anti-mouse IgG2$_b$) and Type IIA

TABLE 1 Descriptive characteristic of 35 canines of various breeds

Breed	Lifespan (years)	Weight range (kg)	Quantity (n)	Sex (n)	Age (years)	Weight (kg)	Neutered
Boerboel	10–12	65–80	1	M	10	39	No
Chihuahua	14–18	1–3	1	F	13	5	No
Chow Chow	10–12	23–34	1	F	10	22	No
Dachshund	12–14	7–14	4	F	8	9	Yes
				M	14	7	Yes
				M	9	6	No
				M	8	7	No
					10 ± 3	7 ± 1	
Dobermann	10–12	29–40	1	F	10	31	Yes
Fox Terrier	10–13	8–10	3	F	13	5	Yes
				F	6	9	Yes
				M	1	--	Yes
					10 ± 5	7 ± 3	
German Pointer	12–14	20–34	1	M	8	46	No
German Shepherd	10–12	34–43	1	F	1	18	No
Greyhound	15–16	24–32	1	F	11	12	Yes
Jack Russell Terrier	13–15	7–12	8	M	6	8	No
				M	10	13	Yes
				M	8	9	No
				M	6	8	No
				M	10	6	No
				M	18	11	Yes
				M	18	7	No
				M	18	5	No
					12 ± 5	8 ± 2	
Labrador Retriever	10–12	25–31	4	F	3	35	Yes
				F	7	30	Yes
				F	7	37	No
				F	10	21	No
					7 ± 3	31 ± 7	
Miniature Pinscher	10–16	3–5	2	F	10	5	No
				M	9	3	Yes
					10 ± 1	4 ± 1	
Mixed breed[a]	–	–	3	M	4	10	No
				M	15	21	Yes
				M	10	28	Yes
					10 ± 6	20 ± 9	
Scottish Terrier	12–13	8–10	2	M	6	8	No
				M	16	6	No
					11 ± 7	7 ± 1	
Toy Pomeranian	12–16	2–3.5	1	F	9	4	No
Yorkshire Terrier	14–16	1–4	1	F	10	3	Yes

Note: Typical parameters from the literature are included for lifespan and weight range. Where applicable, data are expressed as mean ± SD or expressed as the individual value. The typical characteristics of these animals were obtained from Hill's Pet Nutrition (Hill's, 2018), Pedigree (2014), and DogTime (2019).

Abbreviations: F, female; M, male.

[a]Mixed breed contains two animals that are crossed with a German Shepherd and one unknown cross.

(SC71; monoclonal anti-mouse IgG$_1$) fibers. Type IIX fibers were lightly stained by SC71 (Figure 2a). The following day, slides were washed in PBS and incubated for 1 hr at room temperature with fluorescently tagged secondary antibodies (purchased from Jackson ImmunoResearch Laboratories, West Grove, PA) at a dilution of 1:250 in PBS, namely, AMCA goat anti-mouse IgG2$_b$ (catalog number: 115-155-207) and AlexaFluor 488 goat anti-mouse IgG$_1$ (catalog number: 115-545-205). Slides were mounted with Mowiol mounting media containing anti-fade and stored at 4°C until imaging.

Sections were visualized with a fluorescent microscope (Nikon Eclipse 80i; Nikon, Melville, NY) and images acquired at ×10 magnification with a digital camera (Canon EOS 650D; Canon, Tokyo, Japan). Fibers were identified as pure Type I, IIA, or IIX (Figure 2a) and the number of each fiber type was expressed as a percentage of the total number of fibers. For each muscle sample, 676 ± 316 fibers (TB) and 700 ± 280 fibers (VL) were counted. The CSA (in μm^2) of 50 fibers per fiber type were determined for each muscle using the computer program ImageJ (version 1.50e; ImageJ, Bethesda, MD). The image was
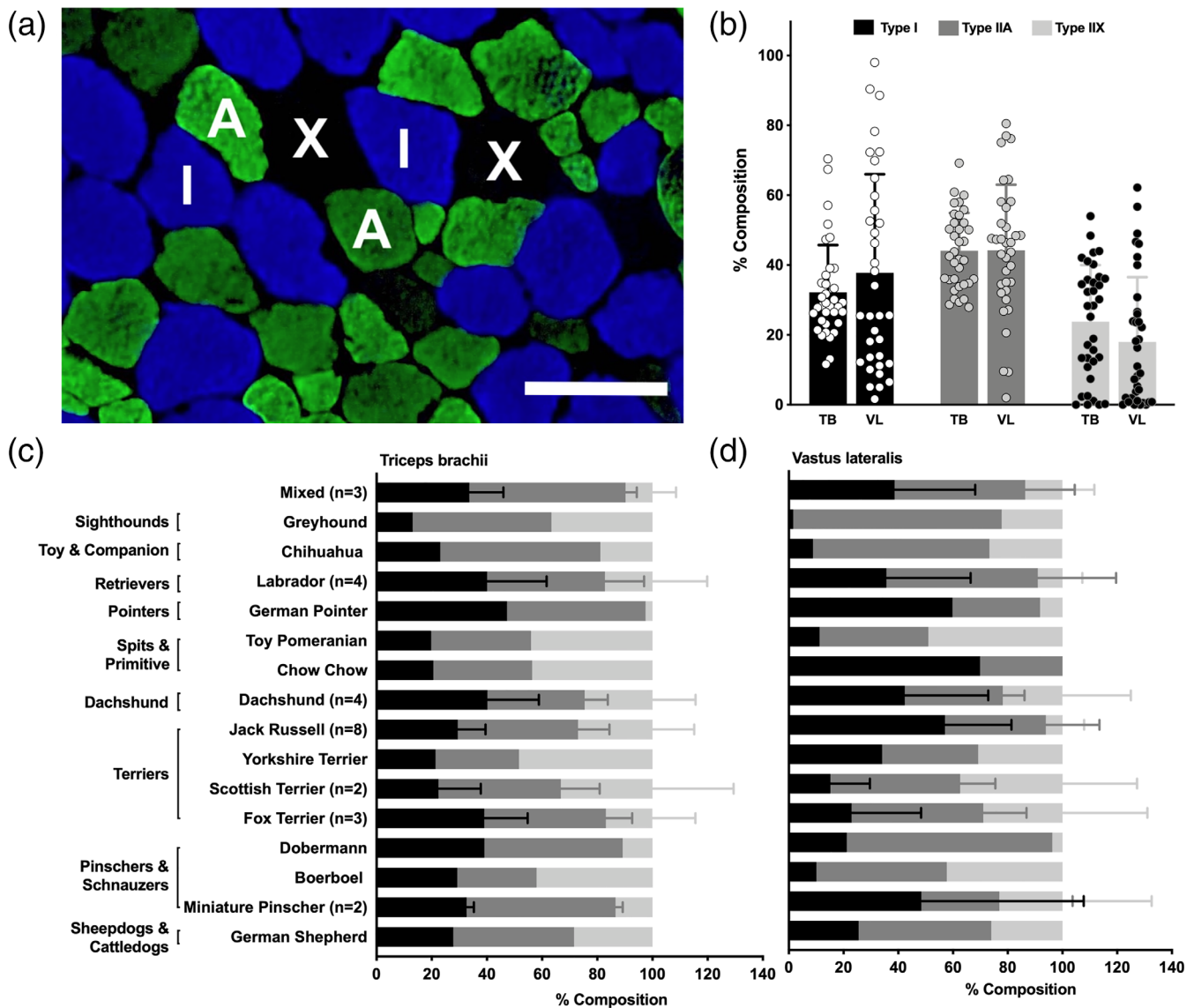

FIGURE 2 (a) Immunohistochemistry of a cross-sectional cut of the *vastus lateralis* (VL) in a dog sample. The blue fluorescence indicates Type I fibers, the bright green fluorescence indicates Type IIA and the lightly stained green indicates Type IIX fibers. The image was captured at ×10 magnification. Scale bar = 100 μm. (b) The fiber type composition (%) of the *triceps brachii* (TB) and VL of 35 samples. The circles represent individual values, the bars represent mean ± SD. Black bars represent Type I fibers, dark gray represents Type IIA, light gray represents Type IIX. (c, d) The fiber type composition (%) in relation to overall total composition in the TB and VL of each breed contained in this study, breeds are further grouped into their respective FCI breed group. Bars represent either mean or mean ± *SD*. Black bars represent Type I fibers, dark gray represents Type IIA, light gray represents Type IIX

divided into five sections and the CSA of 10 randomly selected fibers in each section was determined to ensure that the entire muscle section was represented.

2.5 | Sample preparation for enzyme assays

Muscle samples were homogenized and prepared for enzyme analyses according to Webster et al. (2020), with slight modifications:

Approximately 20 mg wet weight muscle was diluted to a ratio of 1:100 (1 mg muscle to 100 µl buffer) with 0.1 M potassium phosphate buffer, pH 7.3. Following homogenization by hand, the suspension was sonicated on ice. Total protein was determined using the method described by Bradford (1976).

2.6 | Fluorometric enzyme assays

Various enzymes can be used as flux markers of different metabolic pathways. In the present study, the markers of oxidative capacity were citrate synthase (CS) and 3-hydroxyacyl acetyl Co enzyme A dehydrogenase (3HAD), which are marker enzymes of the Kreb's cycle and β-oxidation, respectively. Creatine kinase (CK) and lactate dehydrogenase (LDH) acted as markers of the high energy phosphate pathway and glycolytic capacity, respectively. The above enzyme activities were determined fluorometrically and represent the maximum flux capacity through their respective metabolic pathways (Webster et al., 2020). All reactions were conducted at 25°C with an excitation and emission wavelength of 340 and 460 nm, respectively (Biotek Synergy HT; Biotek Instruments, Winooski, VT). The slope of fluorescence over time was calculated and converted to moles substrate using a NADH or NADPH standard curve. Maximum enzyme activity was expressed as μmol min^{-1} g protein^{-1}.

Enzyme activity data obtained from the dog samples were compared to the enzyme activity of 12 human athletes—6 recreational runners and 6 endurance cyclists (Figure 5). The data were collected in the same laboratory and was used as routine reference points for comparative purposes, for which ethical approval was previously obtained. The comparative human athlete data has been reported in previous studies (Kohn et al., 2011; Van Boom et al., 2022; Webster et al., 2020) and aligns with published literature in humans.

2.7 | Statistical analyses

Because of the low sample size per breed, limited statistical analyses were performed. Where applicable, values are expressed as mean $\pm$ standard deviation and normality determined using the Shapiro–Wilk test (GraphPad Prism version 7, La Jolla, CA). The fiber type, fiber CSA, and enzyme activities between the two muscle groups were compared using the nonparametric Kruskal–Wallis one-way analysis of variance multiple comparison test. Multiple linear regression analyses (SPSS Statistics version 25; IBM) was used to investigate the association between the dependant (fiber type proportions, CSA, and enzyme activities) and the independent variables (breed, sex, age, weight, and neutered status). The relationship between CSA and the weight of the dogs were further analyzed and fitted using a nonlinear model (two-phase decay for line of best fit). Significance was set at $p < .05$.

3 | RESULTS

3.1 | Description of study animals

The descriptive characteristics, typical life span and weight of the 16 dog breeds ($n = 35$) included in this study are presented in Table 1. This was an opportunistic sampling exercise and the number of animals, their sex, and breed could not be controlled for. It is for this reason that some breed groups comprised predominantly of males or females, with some groupings containing a sample size of only one.

3.2 | Fiber type composition

In the TB and VL of all dogs combined (Figure 2b), Type IIA fibers were the predominant fiber type (TB: 44 $\pm$ 11%; VL: 44 $\pm$ 19%) followed by Type I (TB: 32 $\pm$ 14%; VL: 38 $\pm$ 28%) and IIX (TB: 24 $\pm$ 16%; VL: 18 $\pm$ 19%) fibers, respectively. There was no significant difference between the TB and VL with respect to the proportions of Type I, IIA, or IIX fibers. All the fiber types had a large standard deviation, which is apparent from the spread in data from the individual dogs (Figure 2b). The number of hybrid fibers, that is, fibers expressing both MHC I and MHC IIA or MHC IIA and MHC IIX, were negligible with 6 and 5% in the TB and VL, respectively.

Figure 2c,d displays the fiber type composition specific to breed, with each breed grouped according to the FCI breeding group standards. Notably, there was a wide distribution of fiber type proportions between breeds. Breeds with a relatively higher proportion of type I fibers include the German Pointer (VL), Chow Chow (VL), Dachshund (TB and VL), Jack Russell (VL), and Miniature Pinscher (VL). A higher number of breeds had a predominance of Type IIA fibers in either one or both

muscle groups—mixed (TB and VL), Greyhound (TB and VL), Chihuahua (TB and VL), Labrador (TB and VL), German Pointer (TB), Jack Russell (TB), Yorkshire Terrier (VL), Scottish Terrier (TB and VL), Fox Terrier (TB and VL), Dobermann (TB and VL), Boerboel (VL), Miniature Pinscher (TB), and German Shepherd (TB and VL). The only breeds with a higher proportion of Type IIX fibers were the Toy Pomeranian (TB and VL), Chow Chow (TB), Yorkshire Terrier (TB), and Boerboel (TB). Interestingly, the VL and TB muscles from the Toy Pomeranian, Yorkshire Terrier, and Boerboel comprised of a large number of Type IIX fibers (>33%). In contrast, the Labrador (VL), German Pointer (TB and VL), Jack Russell (VL), and Dobermann (TB and VL) seemed to have a low proportion of Type IIX fibers, with no IIX fibers identified in the VL of the Chow Chow. The Greyhound also had an interesting fiber type profile with a very low proportion of Type I fibers identified in both muscle groups.

3.3 | Cross-sectional area

The mean CSA of Type I, IIA, and IIX fibers in the TB and VL are shown in Figure 3A. There was no significant difference in CSA between Type I, IIA, and IIX fibers of the TB and VL. Due to the apparent similar CSA of Type I, IIA, and IIX fibers, they were grouped together for each breed and a mean CSA was determined (Figure 3b). The mean fiber CSA in the TB and VL was $1763 \pm 697 \ \mu m^2$ across all three fiber types. The shaded area of Figure 3b represents the range of CSA from previous human studies conducted in the same laboratory (Kohn et al., 2011; Kohn, Essen-Gustavsson, et al., 2007).

The range of mean CSA in the TB was 1,038–3,898 μm^2. The German Pointer had the largest TB fibers with no other breed having a CSA of greater than 3,000 μm^2. The mixed breed and the Labrador had the second and third largest TB CSA values, respectively. As expected, the Yorkshire Terrier had the smallest TB CSA followed by the Miniature Pinscher.

The range of mean CSA in the VL was 770–2,758 μm^2. The Boerboel had the largest VL CSA followed by the Labrador, mixed breed and Chow Chow. The breeds with the smallest VL CSA were the Yorkshire Terrier, Miniature Pinscher, and Chihuahua.

3.4 | Correlations

Significant relationships ($p < .05$) were found for the following: (a) higher proportion of Type IIA (in TB) fibers in all neutered dogs (regardless of sex); (b) higher proportion of Type I fibers (in VL) in older dogs, corresponding to the concomitant lower proportion of Type IIX fibers in older dogs; and (c) a lower LDH activity in older dogs. Notably, there was a significant positive correlation ($p < .001$) between CSA (in the TB and VL) and the weight of the dogs.

The correlation between the CSAs and the weight of the dogs were further investigated using nonlinear regression (Figure 4). The curve is representative of all the fibers grouped with the VL having a marginally stronger correlation than the TB. Noticeably, the majority of the body weights were below 20 kg, which caused clustering at the lower part of the graph.

3.5 | Oxidative capacity

CS and 3HAD activities are presented as markers of oxidative capacity in the skeletal muscle of the various dog breeds (Figure 5a,b). The mean CS and 3HAD activities of recreationally active and endurance athletes are shown for comparative purposes. There was no significant difference in the CS and 3HAD activities between the TB and VL across the breeds. The CS and 3HAD activity of the dogs appear to be within the range of human activity.

For the various dog breeds, the range of CS activities in the TB were 38–80 $\mu mol \ min^{-1} \ g \ protein^{-1}$, while the range in the VL were similar at 33–66 $\mu mol \ min^{-1} \ g \ protein^{-1}$. The breeds with the highest CS activities were the Greyhound (TB and VL), Chihuahua (VL), Labrador (TB), Dachshund (TB), Scottish Terrier (TB), Fox Terrier (TB), Dobermann (TB and VL), Miniature Pinscher (TB), and German Shepherd (TB). The lowest CS activities were measured for the German Pointer (VL), Chow Chow (TB and VL), Boerboel (VL), Miniature Pinscher, and German Shepherd (VL).

The range of 3HAD activities in the TB and VL were very similar at 29–78 and 28–73 $\mu mol \ min^{-1} \ g \ protein^{-1}$, respectively. The highest 3HAD activities belonged to the Greyhound (TB and VL), Chihuahua (TB), Toy Pomeranian (TB), Dachshund (TB), Scottish Terrier (TB), Dobermann (TB and VL), Boerboel (TB), Miniature Pinscher (TB), and German Shepherd (TB). The lowest 3HAD activities were found in the Labrador (VL), German Pointer (VL), Chow Chow (TB and VL), Scottish Terrier (VL), Boerboel (VL), and Miniature Pinscher (VL).

3.6 | Glycolytic capacity

There were no significant differences in the LDH and CK activities between the TB and VL (Figure 5c,d). Comparatively, the LDH and CK activity of the recreationally active humans were lower than the endurance athletes.
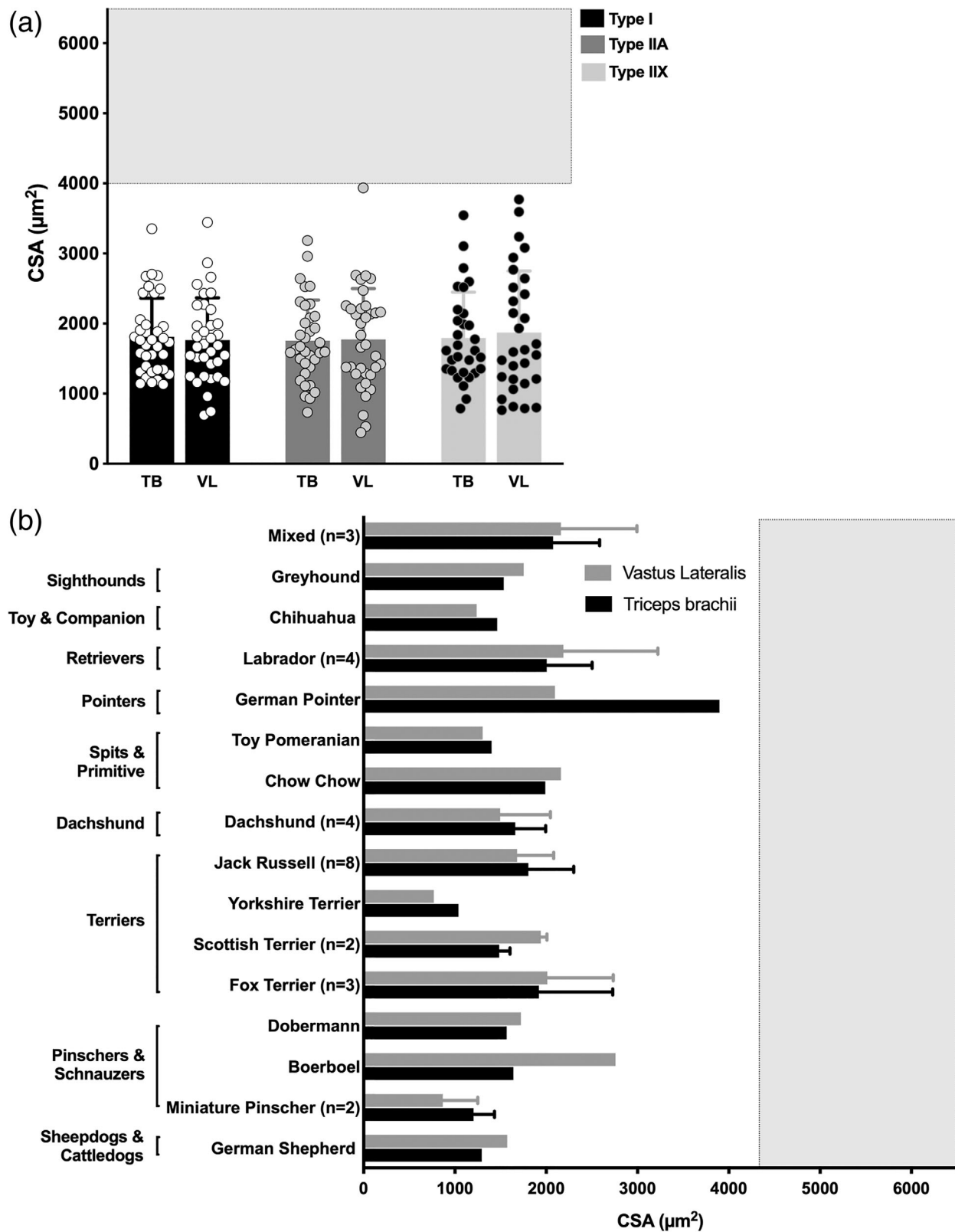
FIGURE 3 (a) The cross-sectional area (CSA) of the *triceps brachii* (TB) and *vastus lateralis* (VL) of 35 dogs. The circles represent individual values, while the bars represent mean ± *SD*. Black bars represent Type I fibers, dark gray represents Type IIA, light gray represents Type IIX. The shaded gray area represents the range of human CSA from previous studies. (B) The CSA (μm²) in the TB and VL of each breed contained in this study. Breeds are further grouped into their respective FCI breed group. Bars represent either mean or mean ± *SD*. Black bars represent TB, gray bars represent VL. The shaded gray area represents the range of human CSA from previous studies (Kohn et al., 2011; Kohn, Essen-Gustavsson, et al., 2007)
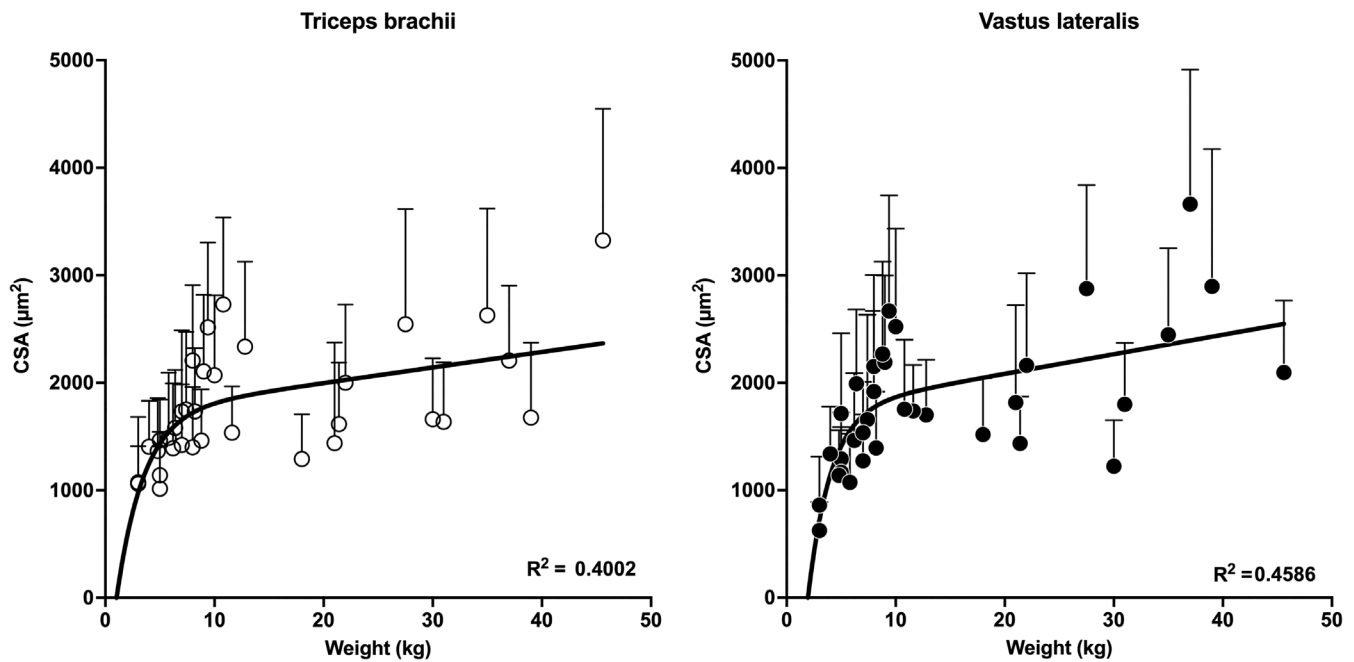
FIGURE 4 The correlations between CSA (μm^2) and animal weight in the *triceps brachii* and *vastus lateralis* of 34 dogs. The circles represent the combined Type I, IIA, and IIX CSA with lines representing the *SD* of an individual animal. The curve represents the nonlinear regression of all fiber types grouped together. One fox terrier had an unknown weight and was excluded from this figure

These human LDH values appear two to three times lower than the dog activities, while the CK activity of the dogs appear to be on par with recreationally active humans.

The LDH activities of the different dog breeds in the TB and VL were similar with a range of 835–2,514 and 891–2,-469 $\mu mol\ min^{-1}\ g\ protein^{-1}$, respectively. It appears that the highest LDH activities were recorded for the Greyhound (VL), Chihuahua (TB and VL), Toy Pomeranian (VL), Chow Chow (TB and VL), Jack Russell (TB), Yorkshire Terrier (TB and VL), and Boerboel (TB and VL). The lowest activities were recorded for the Labrador (VL), German Pointer (TB and VL), Fox Terrier (TB), Dobermann (TB), and Miniature Pinscher (TB and VL).

The activity range of CK was 4,531–8,134 $\mu mol\ min^{-1}\ g\ protein^{-1}$ (TB) and 4,972–7,184 $\mu mol\ min^{-1}\ g\ protein^{-1}$ (VL). The highest CK activities were recorded for the Greyhound (TB and VL), Chihuahua (TB and VL), Labrador (TB), Toy Pomeranian (VL), Dachshund (TB and VL), Jack Russell (TB and VL), Yorkshire Terrier (TB and VL), Boerboel (TB), and German Shepherd (VL). The lowest CK activity belonged to the German Pointer (TB and VL), Chow Chow (TB), Miniature Pinscher (VL), and German Shepherd (TB).

4 | DISCUSSION

This is the first study to investigate muscle fiber type, CSA and muscle metabolism in 16 breeds of dogs. Only a

handful of studies have investigated dog skeletal muscle characteristics, each differing in analyses and research questions. The present study focused on metabolic and structural components of the muscles of these breeds in order to establish a baseline for future research into diseases, such as insulin resistance and Type 2 diabetes.

4.1 | Fiber type composition

In this study, the predominant fiber type was the fast twitch oxidative IIA fibers, followed by Type I and IIX. This finding was evident in both the TB and VL muscle groups and is supported by previous research on dogs, which included muscle groups such as the *semitendinosus*, *longissimus dorsi*, *gluteus medius*, *biceps femoris*, TB, and VL (Acevedo & Rivero, 2006; Braga et al., 2016; Braund et al., 1982; Guy & Snow, 1981; Latorre et al., 1993; McKenzie et al., 2005; Strbenc et al., 2004; Toniolo et al., 2007). Acevedo and Rivero (2006) found that dog muscle fibers expressing MHC IIX were only found in the *latissimus dorsi*, *gluteus medius*, and *semitendinosus* (averaging 8% of the muscle fibers). Type IIX fibers were not found in the TB or *vastus intermedius*. They also found that hybrid IA and IIAX fibers comprised 28% of the total number of fibers across all muscle groups. Toniolo et al. (2007) used gel electrophoresis to determine the percentage of Type IIX fibers in dog muscle, reporting that Type IIX fibers represented on average
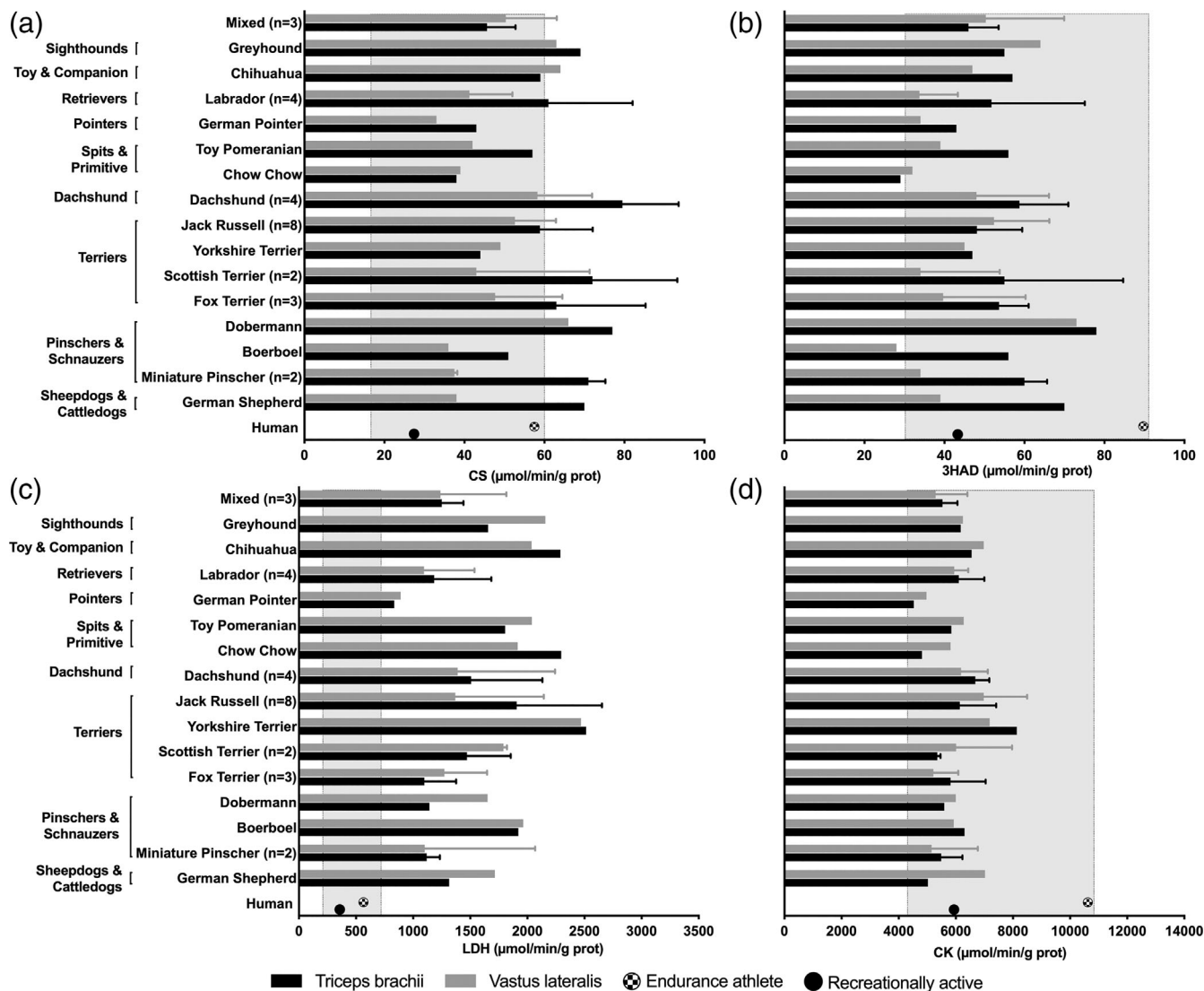

FIGURE 5 (a) Citrate synthase (CS), (b) 3-hydroxyacetyl-coA dehydrogenase (3HAD), (c) lactate dehydrogenase (LDH), and (d) creatine kinase (CK) enzyme activities in the *triceps brachii* (TB) and *vastus lateralis* (VL) of each breed contained in this study. Breeds are further grouped into their respective FCI breed group. Bars represent either mean or mean ± SD. Black bars represent TB, gray bars represent VL. The circles and shaded gray area represents the range of human enzyme activities from previous studies (Kohn et al., 2011; Van Boom et al., 2022; Webster et al., 2020)

22% of the five muscle groups investigated, but not all the muscle groups investigated expressed IIX fibers. The authors concluded that Type IIX fibers are present in most, but not all limb muscles of the dog, while Type IIA fibers were the most abundant fiber type present. The breed of dog known to have some of the best endurance and power in the world, is the Alaskan sled dog for which a few breeds exist. Research showed that the Alaskan Malamute sled dog harbored 40% (range of 30–48%) Type I fibers in their *biceps femoris*, with a high capacity to oxidize fats and glucose (McKenzie et al., 2005). It is, however, unclear what the Type IIA and IIX fiber content was and whether this high percentage is due to an adaptation to exercise training or purely inherent.

In the present study, 24 and 18% of the total number of fibers analyzed were Type IIX from the TB and VL, respectively (Figure 2b–d) and correspond to the study by Toniolo et al. (2007) and Braga et al. (2016). However, the latter study did not distinguish between Type IIA and IIX fibers, relying on an estimation for these fiber proportions. The major limitation of previous studies were that very few fibers were studied in total across all muscle groups (Acevedo study: 1083 fibers; Toniolo study: 247 fibers) that may account for the differences observed in the present study in which a total of ±52,000 fibers were studied. There were limited number of hybrid fibers (IA and IIAX) identified in the present study with only 6% identified in the TB and 5% in the VL. The low

number of hybrid fibers may be due to the difficulty in identifying them through immunohistochemistry, where Type I, IIA, and IIX fibers are clearly visible using the specific antibodies (Figure 2a). Alternatively, the high number of hybrid fibers may be due to methodological differences, especially when relying on ATPase histochemistry.

The proportions of Type I, IIA and IIX fibers varied between the different FCI recognized groups, as well as between the breeds. Overall, most breeds had a higher proportion of Type IIA and Type I fibers. The German Pointer and Dachshund had consistently higher proportions of Type I fibers, corroborating their breed characteristics as endurance hunting dogs—the German Pointer as a Gundog and the Dachshund as a below ground tracker and hunter (FCI, 2019). Breeds with a higher proportion of Type IIA followed by Type I fibers in both muscle groups include the Labrador, Jack Russell, Fox Terrier, Dobermann, and Miniature Pinscher. This fiber type profile is expected for the small, energetic terriers and Miniature Pinscher as well as the Labrador bred for retrieving game and birds (FCI, 2019). However, it is slightly unexpected for the large Dobermann, considered to be a good working and protection dog (FCI, 2019).

The breeds with the highest fast twitch fiber content were the Toy Pomeranian, Yorkshire Terrier, and Boerboel. This matches the apparent powerful and muscular characteristics of the Boerboel, but is somewhat unexpected for the small Toy Pomeranian and Yorkshire Terrier. However, the Toy Pomeranian is descended from the German Spitz, a large working dog from the Arctic, and the Yorkshire Terrier was originally bred to hunt rodents in textile mills and coal mines (FCI, 2019). Therefore, their ancestry may account for their high Type IIA and IIX content. Other breeds with a high fast twitch fiber content (i.e., high proportion of Type IIA and IIX fibers) include the Greyhound, Chihuahua, Scottish Terrier, and German Shepherd. This aligns with the versatile working dog of the German Shepherd, the alert and agile Scottish Terrier and Chihuahua and the bred-for-speed characteristic of the Greyhound (FCI, 2019).

Interestingly, the Greyhound only had 2% Type I fibers, 76% Type IIA, and 22% Type IIX fibers in the VL. This proportion is similar to a previous study on the VL of the Greyhound, reporting 96% Type II fibers (IIA and IIX) and aligns with their high athletic ability as race dogs (Guy & Snow, 1981). No Type IIX fibers were found in the VL of the Chow Chow, but were present in the TB. This was also seen in an individual Dachshund, Jack Russell and Miniature Pinscher and the VL of a Labrador. The lack of Type IIX fibers in these breeds is likely due to the section of the skeletal muscle sample (Delp & Duan, 1996; Gunn, 1978) and may not necessarily be indicative of a total lack of Type IIX fibers in this muscle. Indeed, this hypothesis is confirmed in the analyses of the enzyme activity (Figure 5) in which varying degrees of oxidative and glycolytic capacity are evident for all dogs, including the Chow Chow.

According to Schiaffino and Reggiani (2011), body size plays an important role in determining functional demands on skeletal muscle and, therefore, fiber type distribution. Smaller animals require a higher proportion of fast twitch fibers and a greater shortening velocity to meet the need of essential muscle locomotion for feeding, hunting and reproduction, likely related to their biomechanical properties—such as limb and tendon length. This may account for the seemingly higher proportion of Type IIX and IIA fibers in smaller dog breeds such as the Toy Pomeranian and Yorkshire Terrier.

Although Schiaffino and Reggiani (2011) suggested that larger animals (including humans) predominantly have higher number of Type I and IIA fibers, this theory does not hold true anymore. While the genotype plays an important role in determining stature of an animal, the work conducted by Kohn et al. on various wild animal species with different body sizes (lion, caracal, springbok, black wildebeest, baboon) showed that fiber type composition was not predicted by the weight of the animal (Curry et al., 2012; Dada et al., 2018; Kohn, 2014; Kohn et al., 2011; Kohn, Hoffman, et al., 2007). This finding is also supported by the current cohort of dog breeds where no significant correlation was found between their weight and fiber type. Future studies should therefore also investigate the contractile properties (such as force and shortening velocity) of each breed as this would reveal more information on the potential power of the muscle and whether this links to the fiber type and the breed characteristics.

4.2 | CSA and correlations

The mean fiber CSA determined for this study was $1,763 \pm 697 \mu m^2$ across all fiber types in the TB and VL. There was no difference in CSA between Type I, IIA, and IIX fibers across the 16 breeds, which was also observed in an earlier study by Kuzon et al. (1989). This value is slightly lower than what was reported by Acevedo and Rivero (2006) who investigated the CSA in different muscle groups of the dog. That study reported that the average CSA of Type I and IIA fibers in the TB and *vastus intermedius* was $2,218 \mu m^2$ (I), $1,446 \mu m^2$ (IIA), and $1925 \mu m^2$ (I), $2,805 \mu m^2$ (IIA), respectively. The CSA of Type IIX fibers was not determined in these muscle groups, but amounted to $2,357 \mu m^2$ in the *latissimus dorsi*.

Comparatively, the CSA in the VL of humans were 5,409 μm^2 (I), 5,174 μm^2 (IIA) and 2,968 μm^2 (IIX) (Kohn et al., 2011). In that same study, the CSA of captive lions averaged 2,014 μm^2 (I), 2,005 μm^2 (IIA), and 3,202 μm^2 (IIX), while the caracal had similar CSAs as the lion, even though there is an approximately 10-fold difference in body weight between these two species. The CSA of the dog breeds in the present study were markedly smaller than humans and similar to that of captive lions and a caracal. As stated by Kohn et al. (2011), a smaller CSA does not necessarily mean that the muscle as a whole generates less power, as the total amount of force generated is dependent on other factors, such as the number of muscle fibers activated, fiber proportions, and ATP supply. However, the present study did not investigate the skeletal muscle power production in the various breeds of the dog and should be investigated in future studies.

Significant relationships were found between the neutered status of the dogs and the proportion of Type IIA fibers (in the TB) as well as with age (in the VL)—older dogs having a higher proportion of Type I fibers, lower proportion of Type IIX fibers and lower LDH activity. An early study by Braund et al. (1982) found that there was no difference between fiber type proportions with increasing age and weight in different breeds of dogs, however there were other morphological indications of aging and sarcopenia in the muscle. Age-related changes in the structure and function of skeletal muscle has been extensively studied in humans with the natural process of aging playing a role regardless of the nutrition, health, and physical activity levels of the individual (Larsson et al., 2019). Therefore, the effect of age may be playing in a role in the current study, however, it is difficult to draw definite conclusions due to the inherent variability of the samples as well as the reliance on record keeping and owner knowledge at the veterinary hospitals where samples were collected.

There was a significant positive correlation between CSA (in the TB and VL) and the weight of the dogs (Figure 4). Therefore, the larger the dog, the larger the CSA of the muscle fibers. This supports previous findings, with dogs greater than 15 kg having a larger mean muscle fiber diameter compared to dogs below 15 kg (Braund et al., 1982). However, it would have been better to correlate muscle mass with CSA, as body weight itself includes too many factors (such as fat, organ size, etc.) that can skew the results. Previous studies using different wildlife species with varying body weights did not show any correlation with fiber CSA (Curry et al., 2012; Kohn, 2014; Kohn et al., 2011). The relationships found in the present study may therefore be an intraspecies (between breeds) phenomenon. What is not well understood is whether muscle fiber number is different between species, and if

so, conserved within a species. This is quite challenging to determine as it will involve analyzing the whole muscle.

4.3 | Oxidative capacity

CS and 3HAD act as markers of flux through the Kreb's cycle and β-oxidation, respectively, with a higher activity indicating a greater flux through the respective pathway, resulting in a greater yield of ATP for muscle contraction. The mean CS and 3HAD activities for the TB and VL were similar to one another. The range of recreationally active and endurance human athletes were included in the figures for comparative purposes. Endurance athletes have nearly twice the CS and 3HAD activity of recreationally active humans (Schiaffino & Reggiani, 2011). The mean CS activity across the dog breeds was similar to that of endurance athletes, while the 3HAD activity was closer to that of recreationally active humans (Kohn et al., 2011; Webster et al., 2020). In comparison to wild animals, the dog has higher CS and 3HAD activity than the lion and caracal (Kohn et al., 2011), but lower activity than antelope species (Kohn, 2014). This finding indicates that dogs, overall, have an inherent high oxidative capacity in comparison to human endurance athletes, but not as high as antelope (such as the blesbok and springbok).

Across the different breeds, a wide range in CS and 3HAD activities were found. The Dobermann and Greyhound had consistently high CS and 3HAD activities across both muscle groups, while the Dachshund, Scottish Terrier, and Miniature Pinscher had high activities in only the TB. This would indicate that these breeds have a higher oxidative capacity and are able to more efficiently utilize the Kreb's cycle and β-oxidation pathways to produce ATP. The higher oxidative capacity does match the function of the above mentioned breeds, except for the Dobermann, which is not considered an endurance type breed, but rather a powerful breed (FCI, 2019). However, the Dobermann did have a higher proportion of Type I and IIA fibers which would indicate a higher oxidative capacity and aligns with its enzyme activity profile.

The Chow Chow had consistently low CS and 3HAD activities in the TB and VL, while the German Pointer, Boerboel, and Miniature Pinscher had low activities in only the VL. This would indicate that these breeds have lower oxidative capacity that is closer to a recreationally active human. Interestingly, the Miniature Pinscher had a high oxidative capacity in the TB, but low activity in the VL—and these differences are seen throughout this study in the different breeds. This is not uncommon and

has been found in previous studies with different muscle groups having different fiber type distribution and metabolic activity (Acevedo & Rivero, 2006; Delp & Duan, 1996; Gunn, 1978). The Boerboel is considered a strong, muscular dog and it was expected that, overall, it would have had a lower oxidative capacity (FCI, 2019). However, this was only found in the VL muscle. In contrast, the German Pointer is considered an endurance Gundog and would be expected to have a higher oxidative capacity as it also had one of the highest proportions of Type I fibers in their muscles. As for the Boerboel, high oxidative capacity was only found in the TB. The oxidative capacity of the Chow Chow matched the low proportion of Type I fibers observed in Figure 2.

4.4 | Glycolytic capacity

CK and LDH activities act as markers of the anaerobic capacity of the muscle, which is the ability to produce ATP rapidly without the need for oxygen. The mean LDH activity of the dog was three times higher than that of endurance trained humans, while the mean CK activity was similar to recreationally active humans (Kohn et al., 2011; Webster et al., 2020). In comparison to antelope species, the dog has a lower LDH activity and a similar CK activity (Kohn, 2014), but felids (lion and caracal) have much higher LDH and CK activities (Kohn et al., 2011). This would indicate that the dog has a greater ability to convert pyruvate to lactate under anaerobic conditions compared to endurance athletes, but a lower ability than antelope or felid species. This high glycolytic capacity is quite surprising considering the highly oxidative capacity and high proportions of Type I and IIA fibers described earlier. However, the muscle fiber type does not necessarily define the oxidative or glycolytic capacity of the skeletal muscle, with other factors, such as exercise training and genetics playing a significant role (Kohn, Essen-Gustavsson, et al., 2007). The training and health status of the dogs included in this study were unknown at the time of euthanasia and could have potentially influenced the results.

The different breeds had a variety of CK and LDH activities. Consistently high CK and LDH activities were found in the Chihuahua and Yorkshire Terrier in both muscle groups. High CK and LDH activities in the VL were recorded in the Greyhound and Toy Pomeranian, and high activity in the TB was found in the Jack Russell and Boerboel. The Chihuahua, Yorkshire Terrier, and Toy Pomeranian are very small, energetic dogs with limited endurance which may account for the higher glycolytic capacity in these breeds (FCI, 2019). In addition, this high glycolytic capacity supports the higher proportion of Type IIA and IIX fibers seen in these breeds. The Greyhound has a high glycolytic and oxidative capacity which highlights the athletic ability of this sighthound and aligns with the high proportion of Type IIA fibers. The high glycolytic capacity was expected in the strong Boerboel.

The breeds with generally lower CK and LDH activities were the German Pointer in both the TB and VL, and the VL of the Miniature Pinscher. Interestingly, the German Pointer had a low oxidative and glycolytic capacity overall compared to the rest of the breeds. This low glycolytic ability does align with the supposedly high endurance capabilities of this breed (FCI, 2019) as well as the low proportions of Type IIX and IIA fibers found in this study. Similarly, the VL of the Miniature Pinscher had low glycolytic and oxidative capacities.

4.5 | Limitations of the study

Muscle sampling was opportunistic—thus, the breed, sex, and age of the samples could not be controlled for or anticipated. This resulted in many of the breeds having only one or two study animals with males and females being grouped together. Therefore, this study is descriptive in nature due to the limited sample size. Small sample sizes are not uncommon in this type of research (Acevedo & Rivero, 2006; Strbenc et al., 2004) with the novelty of this study being the diversity of breeds investigated. A second limitation was that the clinical reasons for euthanasia of the animals were not disclosed to the researcher. Many of the animals were also older than 10 years, and as a result, "old age" was often recorded as the reason for euthanasia. This may have implications on the results, specifically the metabolism of the muscle and fiber CSA.

5 | CONCLUSION

In conclusion, this study determined muscle fiber composition and metabolic profiles in different breeds of dogs and lay the foundation for future research to determine the role of these parameters in diseases, such as insulin resistance and diabetes. Type IIA fibers were shown to be the most prevalent fiber type in the breeds analyzed, followed by Type I and then Type IIX. However, there was variability across the different breeds, which may be attributed to either genetics, function, lifestyle or all of these factors. In addition, the dogs analyzed in this study seemed to have both high oxidative and glycolytic capacity, which links to their higher proportions of Type I and IIA fibers, confirming what was found in previous studies

(Acevedo & Rivero, 2006; Snow et al., 1982; Strbenc et al., 2004; Toniolo et al., 2007). Notably, the specific breeds with a higher proportion of Type I fibers do not necessarily have the highest oxidative capacity. Muscle fiber type does not solely dictate the metabolic properties of the muscle (Kohn, Essen-Gustavsson, et al., 2007) with factors such as function and lifestyle of dogs potentially accounting for their high oxidative capacity. Of course, genetics of the different breeds should not be ruled out, but would require a more in-depth approach of analyses. The fiber CSAs of the dogs were similar to that found in a previous study (Acevedo & Rivero, 2006) and were markedly smaller than humans, but similar to lion and caracal fiber CSA (Kohn et al., 2011). Finally, there was a significant positive correlation between the weight of the dogs and their CSAs, with heavier dogs having a greater CSA.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ORCID

Kathryn M. van Boom https://orcid.org/0000-0003-1483-9061
Tertius A. Kohn https://orcid.org/0000-0003-4152-6319

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