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Expiratory Dysfunction in Young Dogs with Golden Retriever Muscular Dystrophy

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Abstract

Respiratory disease is a leading cause of morbidity in people with Duchenne muscular dystrophy and also occurs in the golden retriever muscular dystrophy (GRMD) model. We have previously shown that adult GRMD dogs have elevated expiratory flow as measured non-invasively during tidal breathing. This abnormality likely results from increased chest and diaphragmatic recoil associated with fibrosis and remodeling. Treatments must reverse pathologic effects on the diaphragm and other respiratory muscles to maximally reduce disease morbidity and mortality. Here, we extended our work in adults to younger GRMD dogs to define parameters that would be helpful in preclinical trials. Tidal breathing spirometry and respiratory inductance plethysmography were performed in GRMD dogs at approximately 3 and 6 months of age, corresponding to approximately 5–10 years in DMD, when clinical trials are often conducted. Expiratory flows were markedly elevated in GRMD versus normal dogs at 6 months. Values increased in GRMD dogs between 3 and 6 months, providing a 3-month window to assess treatment efficacy. These changes in breathing mechanics have not been previously identified at

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Contributions of each author:

Hawkins: Creation of protocol for pulmonary function testing, off-site supervision of technicians collecting data, analysis of data, primary manuscript preparation.

Bettis: Collection of respiratory data and supervision of care of subjects.

Kornegay: Review of protocol for pulmonary function testing, provision of subjects, on-site supervision of technicians, review of data and manuscript.

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such an early age. Expiratory flow measured during tidal breathing of unsedated young GRMD dogs could be a valuable marker of respiratory mechanics during preclinical trials.

Keywords

Duchenne muscular dystrophy (DMD); Golden retriever muscular dystrophy (GRMD); spirometry; respiratory inductance plethysmography; pulmonary function test; peak expiratory flow

1.0 INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked recessive disease that affects approximately 1 in 4000–6000 boys, making it the most common form of muscular dystrophy [1]. Mutations in the *DMD* gene cause loss of the protein dystrophin, which normally buttresses the muscle cell membrane. Resulting membrane instability leads to repeated cycles of skeletal and cardiac myofiber necrosis and regeneration, with muscle being replaced over time by connective tissue and fat [2]. Muscles required for ventilation are severely affected, with marked changes in the diaphragm [3]. Respiratory failure results from progressive ventilatory compromise, often complicated by bronchial infection or pneumonia [3,4].

Preclinical studies of DMD disease pathogenesis and treatment have principally been conducted in genetically homologous murine and canine models. The mdx mouse has been extremely valuable to establish proof of concept for disease mechanisms and treatment efficacy. However, mdx mice have a relatively mild disease and findings have not consistently translated to humans [5,6]. The most studied canine condition was originally reported in golden retrievers and termed golden retriever muscular dystrophy (GRMD) [7,8]. Dystrophic dogs have progressive skeletal and cardiac muscle disease in keeping with DMD. The initial clinical course of GRMD parallels that of DMD, on an accelerated time scale, with 3 to 6 months of age roughly corresponding to 5–10 years in DMD [9]. Numerous preclinical trials have been conducted in GRMD dogs over this age period [9].

Respiratory insufficiency contributes greatly to the morbidity of muscular dystrophy and, along with cardiomyopathy, is a primary cause of death [3,10]. In the absence of ventilator support, death from complications associated with respiratory muscle weakness would occur in most patients by 20 years of age [11]. To better utilize GRMD in preclinical studies of respiratory disease, we have studied the pathophysiology of their respiratory impairment and sought to establish objective markers of disease progression [12,13]. The most effective treatments for muscular dystrophy will need to be initiated prior to extensive muscle fibrosis and other irreversible adaptations. Therefore, it is particularly important to determine how early in life abnormalities in respiration can be measured in GRMD dogs, especially over 3 to 6 months of age when preclinical trials are typically conducted [6,9].

Our laboratory previously reported that unsedated, adult GRMD dogs (median age of 48 months) have elevated expiratory flow as measured non-invasively by tidal breathing spirometry and respiratory inductance plethysmography (RIP) [12]. We also identified a

brief period of paradoxical abdominal breathing within each breath, not measured by phase angle, in some affected dogs. A separate study showed that altered breathing mechanics in GRMD dogs were associated with severe changes in ventilatory capacity by approximately 1 year of age [13].

The purpose of this study was to determine if unsedated GRMD dogs as young as 2–3 months of age would show similar elevations in expiratory flow or periods of abdominal paradox within each breath and whether these changes would progress over the critical 3 to 6 month age period.

2.0 MATERIALS AND METHODS

2.1 Animals

Golden retriever-cross dogs from a GRMD colony at Texas A&M University were used and cared for according to principles outlined in the National Resource Council Guide for the Care and Use of Laboratory Animals. Procedures were approved by the Texas A&M Institutional Animal Care and Use Committee through protocol 2015–0110 (Standard Operating Procedures – Canine X-Linked Muscular Dystrophy). Dogs were classified as having GRMD based on elevation of serum creatine kinase (CK) as newborns and subsequently developing characteristic signs of the disease. The GRMD genotype was confirmed by PCR [14]. Both hemizygous males and homozygous females were included, as our skeletal muscle functional testing has not shown gender differences [15]. Controls were generally drawn from the same litters and included both normal male dogs and female hemizygous carriers without phenotypic disease.

In our laboratory, GRMD and control dogs underwent respiratory function testing by spirometry and RIP as part of several different preclinical studies over a 4 year period with dogs in a variety of body positions (lateral, sitting, standing, and/or sternal recumbency). Spirometric and RIP data were most often available for GRMD dogs less than 1 year of age when they were positioned in lateral recumbency. Accordingly, for this study, we included only dogs from which spirometric or RIP data, or both, were of acceptable quality and had been collected with the dog in lateral recumbency. Dogs were excluded if they had received any specific treatments for GRMD.

2.2 Instrumentation

Unsedated dogs were instrumented for RIP with a commercially available, telemetric, jacketed system (Data Sciences International, St. Paul, MN) prior to beginning data collection as previously described [12]. Briefly, elastic inductance bands were incorporated within a spandex shirt. The bands were positioned around the thorax and cranial abdomen with sufficient tension to move with respirations while avoiding restriction of wall movements. A loose fitting, outer mesh jacket held wires and the telemetric device that transmitted data to the processing computer (Fig. 1A). Following instrumentation, dogs were acclimated to the procedures room for at least 5 minutes. Activity and noise were minimized.

Dogs were restrained in lateral recumbency on a padded table. The technician restraining the dog held the distal limbs while resting one arm lightly over the neck and the other over the hip, to avoid interference with breathing movements. Another technician calmed the dog verbally or with stroking, as needed. If at any point, the dogs struggled against the restraint or had rapid respirations (>60 breaths/minute), they were released and further calming was attempted. Procedures were aborted in dogs that could not remain calm for several minutes of data collection.

2.3 Tidal breathing spirometry

Tidal breathing spirometry was performed as previously described using a rigid, plastic, anesthetic facemask for dogs (Surgivet, Smiths Medical, ASD, Inc. St. Paul, MN; Fig. 1B) [12]. A modification of the facemask was needed for young dogs due to leakage when using the standard rubber gasket included with these facemasks. Vinyl from an examination glove was stretched tightly across the facemask and a small opening created for passing over the muzzle. The mask was attached to a pneumotachograph (Model 3700, Hans Rudolph, Inc. Shawnee, KS), which was connected to a pressure transducer (DP45, Valdyne, Northridge, CA). The signal was collected for analysis using a commercial physiology software platform (Ponemah Physiology Platform 4.90-SP2, Data Sciences International, St. Paul, MN) with a sampling rate of 500 Hz. Calibration of the pneumotachograph was performed prior to data collection.

Spirometry data were analyzed using the physiology software platform and similar criteria as previously published [12,16–19]. Breaths with artifacts, such as from motion or panting, were eliminated based on visual examination. Additional breaths were eliminated if the respiratory rate exceeded 60 breaths/minute or the difference between inspiratory and expiratory volumes exceeded 5%. Quantitative values from acceptable breaths were averaged for comparisons between dogs. A minimum of eight acceptable breaths were required for inclusion in the study. Values that were measured or calculated are listed in Table 1.

2.4 Respiratory inductance plethysmography

Data were collected from the RIP bands concurrently with spirometry. Breaths from the RIP bands were analyzed as described for spirometry. Phase angle measurements of acceptable breaths were also averaged for comparisons between dogs. Tracings were visually examined for a brief period of abdominal paradox within each breath, as has been described in some adult dogs [12].

2.5 Statistical analysis

Data were not normally distributed and are described as median (25th/75th percentile), unless otherwise noted. Mann-Whitney rank sum test was used to compare data from GRMD and control dogs. $P < 0.05$ was considered significant. Statistical analyses and graph creation were performed using SigmaPlot software version 11.0 (Systat Software, Inc., San Jose, CA).

3.0 RESULTS

3.1 Subjects

The study group was comprised of 23 GRMD and 11 control dogs. Data were grouped by age at time of collection into ranges of 2–3 months, 5–7 months, and adults (> 1 year) for comparisons. Data from 3 GRMD dogs were collected at both 2–3 and 5–7 months of age, and one of these dogs was tested again as an adult (12.1 months).

There were 11 GRMD dogs (4 males, 7 females) in the 2–3 month group, with a median body mass of 5.4 (4.2/6.8) kg. Activity of control dogs precluded data collection by spirometry or RIP from any dog at this age despite multiple attempts, even considering other body positions.

The 5–7 month group included 7 GRMD dogs (4 males, 3 females) and 9 controls (7 males, 2 females). The GRMD dogs had lower body masses (median of 11.2 (9.9/12.7) kg) than controls (median of 17.3 (14.5/17.8) kg), ($p=0.001$).

There were 5 adult GRMD dogs (3 males, 2 females), with a median body weight of 19.3 (16.0/23.4) kg, and 2 female control dogs with body weights of 24.2 and 26.1 kg. The median age of the adult GRMD dogs was 45.4 (range, 12.1 – 112) months, while the 2 control dogs were 45 and 81 months old.

3.2 Spirometry

The absence of a 2–3 month control group precluded comparisons with GRMD dogs at this age. Marked differences were seen when data from 5–7 month GRMD dogs were compared to the 5–7 month controls (Table 1; Fig. 2). Dogs with GRMD had a nearly two-fold greater PEF:PIF than controls (2.46 compared with 1.36; $p<0.001$). Their EF50:IF50 was also approximately double that of control dogs. Additionally, the decline of expiratory flow between peak and 50% of tidal volume as measured by PEF:EF50 was less pronounced compared with control dogs ($p=0.040$). The increased PEF:PIF resulted from greater expiratory flow as measured absolutely (PEF; $p=0.040$), or when adjusted by body weight (PEF/kg; $p=0.001$). Dogs with GRMD had a significantly shorter ratio of expiratory to inspiratory time (Time Exp:Time Insp; $p<0.001$). Although neither of these values were different when compared independently, dogs with GRMD showed a trend toward shorter expiratory time ($p=0.054$).

The 2 adult control dogs had a PEF:PIF of 0.94 and 1.43, while the median value for the 5 adult GRMD dogs was 2.61 (range, 2.19–3.23; Fig. 2A). Similarly, for EF50:IF50, control dogs had values of 0.90 and 1.52, compared with dogs with GRMD (2.94; range, 2.12–3.65; Fig. 2B). Low dog numbers precluded statistical analysis.

The elevation in expiratory flow ratios identified in GRMD dogs progressed between 2–3 months and 5–7 months of age, with the median PEF:PIF being significantly higher in the older dogs (2.46 versus 2.07; $p=0.011$). Analogous higher EF50:IF50 values were seen in the older dogs (2.54 versus 2.11; $p=0.026$), and their ratio of expiratory to inspiratory time was lower than in the 2–3 month group (0.55 versus 0.76; $p=0.004$). Significant differences

were not found between the 5–7 month and adult GRMD dogs ($n=5$) for PEF:PIF (median 2.46 compared with 2.61; $p=0.432$) or EF50:IF50 (median 2.54 compared with 2.94; $p=0.073$).

All 3 of the GRMD dogs tested at both 2–3 months and 5–7 months showed an increase in PEF:PIF with age (dog 1: 2.12 to 2.44; dog 2: 2.24 to 2.79; and dog 3: 1.77 to 2.52; Fig. 3A). Dog 3 had a third measurement as an adult, with the value of 2.19 falling between the two prior measurements. Similar patterns were present for EF50:IF50 (Fig. 3B).

3.3. Respiratory Inductance Plethysmography

Data from RIP confirmed the spirometric findings of elevated PEF:PIF and EF50:IF50 in GRMD dogs compared with control dogs, and the greater degree of elevation at 5–7 months compared with 2–3 months in GRMD dogs. The GRMD dogs at 5–7 months had a median PEF:PIF of 2.27 (1.68/2.37) compared with 1.33 (0.98/1.44) in the 5–7 month control dogs ($p=0.002$). The median EF50:IF50 values were 2.56 (2.12/2.73) and 1.21 (1.02/1.55), respectively ($p=0.002$). The elevations in PEF:PIF and in EF50:IF50 were greater in the 5–7 month GRMD dogs compared with those at 2–3 months of age ($p=0.009$ for both). The PEF:PIF of the 2–3 month GRMD dogs was 1.58 (1.51/1.67), and the EF50:IF50 was 1.87 (1.53–1.93).

Adult GRMD dogs ($n=4$) had greater PEF:PIF (2.55; range, 2.26–2.90) and EF50:IF50 (2.70; range, 2.15–3.20) than those for the two control dogs (PEF:PIF, 0.97 and 1.25; EF50:IF50, 0.79 and 1.30).

No differences were found in phase angle between GRMD and control dogs at 5–7 months. None of the individual dogs showed paradoxical breathing or brief periods of abdominal paradox within each breath. Of the adult GRMD dogs, one had paradoxical breathing with a phase angle of -47.6 degrees and one dog showed brief periods of abdominal paradox within each breath.

4.0 DISCUSSION

This study was primarily motivated by a need to identify markers of respiratory disease that progress between the ages of 3 months and 6 months, the age period typically used in GRMD preclinical trials. In particular, we wished to establish whether abnormalities in expiratory flow identified previously using non-invasive methods in unsedated adult GRMD dogs could be detected at 2–3 months of age and whether these changes would be more severe at 5–7 months. The excitable nature of normal/carrier 2–3 month old dogs precluded their assessment and comparison to GRMD at this age. However, several differences were seen at 5–7 months, with peak expiratory flow, expiratory flow at 50% of tidal volume, peak expiratory flow/kg body weight, and the ratios of PEF:PIF and EF50:IF50 all being higher in GRMD versus control dogs. Importantly, the values for PEF:PIF and EF50:IF50 increased in GRMD dogs between 2–3 and 5–7 months, suggesting they could be used to document respiratory disease over this period during preclinical trials. Interestingly, based on limited testing of adult GRMD dogs, respiratory abnormalities seemed to stabilize beyond 6 months of age, consistent with the pattern of disease progression seen in the limbs [20].

With preclinical trials, it is important to identify a primary outcome measure on which to base treatment effect. In this context, the ratio of PEF:PIF is the most relevant and compelling measure because it is normalized for variables between individual dogs beyond body weight, such as chest conformation and tidal volume. Moreover, in our study, this ratio also clearly distinguished GRMD and control dogs at 5–7 months, with little to no overlap in values. Most importantly, there was also a measurable increase in PEF:PIF over the range in age of approximately 3 to 6 months that is typically used in GRMD preclinical trials.

Beyond identifying statistical differences between populations in natural history studies, data should be analyzed to determine group sizes that would be necessary provide adequate statistical power for the design of future preclinical trials [21]. Power analyses for t-tests were performed using data from the 3 GRMD dogs that had PEF:PIF measurements available at both 2–3 and 5–7 months of age. The mean difference between time points was 0.54 with a standard deviation of 0.215. A study population of 4 dogs per group (treated and untreated GRMD dogs) reaches a power of 0.839 (alpha 0.050) for treatment begun at 2–3 months of age that halts the increase in PEF:PIF. Alternatively, a study population of 11 dogs per group reaches a power of 0.800 for treatment that blunts the increase in PEF:PIF by 50%.

While these preliminary data support the potential use of PEF:PIF in treatment trials, additional factors deserve consideration. For instance, treatments that could reverse damage already present by 2–3 months would increase the difference between treated and untreated dogs, reducing the number of dogs needed to demonstrate benefit. More likely, technical issues impacting the collection of usable data would necessitate the inclusion of extra dogs. As discussed further with limitations, collection of data was not always successful even in GRMD dogs at 2–3 months of age.

The elevation of expiratory flow at 50% tidal volume with both spirometry and RIP indicates that the abnormal breathing pattern continues through expiration versus occurring solely at the onset of the expiratory phase. This, and other findings, are consistent with those of Mead, et al. [13], who documented a change in GRMD expiratory breathing mechanics evidenced by a marked decline in the diaphragm's contribution to tidal breathing between 4–6 and 9–18 months [13]. While they did not report expiratory flow data, a hyperactive expiratory push was seen in anesthetized GRMD dogs at 6–18 months upon administration of doxapram, a centrally-active stimulant of respiratory drive given to mimic high demand. This expiratory push decreased end expiratory volume and loaded the diaphragm for greater recoil to facilitate the next inspiration, with a resulting increase in tidal volume. That overall mechanism is in contrast with normal physiology whereby tidal volume is increased through an increase in end inspiratory volume. The mechanism of decreasing end expiratory volume for enhancing ventilation has been identified in people during intense exercise [22,23], and may be augmented by diaphragmatic stiffening in GRMD. Mead et al [13] showed that the weakened diaphragms of 6–18 month old GRMD dogs become shorter, thicker, and severely fibrotic secondary to deletion of sarcomeres, increased collagen in the extracellular matrix, and increased cross-sectional area.

The elevated expiratory flow in GRMD dogs also might result, more broadly, from greater recoil of the lung, chest wall, and diaphragm. In addition to the increased stiffness of the diaphragm, GRMD dogs have increased collagen fiber deposition in the intercostal musculature [13], and within the lung surrounding alveoli [24]. Fibrosis and muscle atrophy have been reported in GRMD dogs as young as 2 months of age, with the diaphragm among the muscles most affected [25,26].

Asynchronous (paradoxical) expansion of the thorax and abdomen is detected by RIP through independent stretching of the two inductance bands. In healthy animals, both bands are expanded concurrently during inspiration. Expansion of the abdominal band results from diaphragmatic contraction, with associated caudal displacement of the abdominal organs. Asynchrony between the thorax and abdomen is measured by phase angle expressed in degrees and calculated by dividing the time difference between the peaks of the chest and abdominal band signals by the cycle time and multiplying by 360. Perfect synchrony or absolute asynchrony are identified by phase angles of 0 and 180 degrees, respectively. With weakness of the diaphragm, negative intra-thoracic pressure created by the intercostal and accessory muscles during inspiration can result in cranial (paradoxical) displacement of the diaphragm, reducing lung volume and ventilation and resulting in a negative phase angle.

We previously demonstrated episodes of paradoxical breathing with RIP in adult GRMD dogs, even though their phase angles were normal [12]. The duration of paradox was relatively brief compared with the full duration of a single breath, and the peak expansion of the bands during the paradoxical motion was less than the peaks generated by the synchronous motion. Therefore, the phase angle calculation did not capture the asynchrony. Changes in phase angle were not found in anesthetized GRMD dogs following stimulation with doxapram as reported by Mead, et al [13]. None of the younger GRMD dogs in the current study showed changes in phase angle or brief periods of paradoxical breathing. Consistent with our earlier study, abnormalities in synchrony were noted in two of the five adult GRMD dogs.

Using a portable system for performing RIP in people, investigators have calculated an index of labored breathing from an algorithm considering phase and the magnitude of input from each band [27]. In particular, patients with neuromuscular disease have been shown to use variable breathing pattern strategies to balance energy expenditure and gas exchange [28,29]. In light of their abnormal expiratory patterns, GRMD dogs likely also employ differing strategies, depending on variables such as disease severity, body size and structure, and demands for oxygenation.

Pulmonary function testing in awake dogs carries distinct advantages over methods requiring anesthesia or sedation in that studies can be repeated over short time intervals and there is no risk of drugs influencing disease progression, reducing respiratory drive, or causing adverse events. Importantly, for our measures of expiratory flow, the influence of increased recoil is likely best identified during the tidal breathing of normal wakefulness. However, our inability to collect data from unsedated 2–3 month old control dogs, due to their excitable nature and rapid respiratory rates outside of our quality control criteria, was a major limitation of this study. Excitability also complicated assessment of younger GRMD dogs.

Over and above the 11 dogs reported, 3 were excluded due to respiratory rates exceeding 60 breaths/minute, potentially creating a selection bias towards a more severe phenotype. The relatively low number of dogs was already an inherent limitation that is present with nearly all large animal model preclinical trials.

Difficulties in collecting data were compounded by other practical and technical issues. In principle, conditioning dogs to the testing procedure could have lessened their excitability, and pre-screening of candidate dogs could have allowed for selection of more stoic dogs. Although all dogs in the colony have regular human interaction and exercise periods, normal dogs are handled less frequently, probably making them more likely to resist being held. From a technical standpoint, the relatively short muzzles of younger dogs sometimes prevented us from maintaining a tight seal around their nose and mouth with the spirometry mask. Resulting air leakage probably led to differences in inspiratory and expiratory tidal volumes and exclusion of spirometry results from five GRMD dogs, although RIP data collected at the same time were acceptable and reported. Notably, spirometry and RIP recordings were made by technicians at Texas A&M but analyzed later at another site. Adequate tracings likely could have been obtained in some excluded dogs had timely feedback been feasible, with the opportunity to adjust the collection system and record additional breaths.

5.0. CONCLUSIONS

Strong evidence is presented that elevated expiratory flow during tidal breathing, as reported in adult GRMD dogs, is present at 5–7 months of age; and, that there is a measurable progression in PEF:PIF between 2–3 months and 5–7 months of age. These findings support the potential value of PEF:PIF as a marker of respiratory disease for GRMD preclinical trials typically done over this period. Based on the limited longitudinal studies done in adult GRMD dogs, expiratory flow elevations appear to stabilize beyond 5–7 months, consistent with the timeline of limb skeletal muscle function.

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ABBREVIATIONS

GRMD	Golden retriever muscular dystrophy
PEF	Peak expiratory flow
PIF	Peak inspiratory flow
EF50	Expiratory flow at 50% tidal volume

IF50	Inspiratory flow at 50% tidal volume
Time Exp	Expiratory time
Time Insp	Inspiratory time

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Highlights

- Dogs with GRMD have elevated tidal expiratory flow at 5–7 months of age
- Expiratory flow increases between 2–3 and 5–7 months of age in dogs with GRMD
- Paradoxical abdominal motion was not noted in young dogs with GRMD at rest
- Pulmonary function testing is difficult in unsedated dogs at 2–3 months of age

Fig. 1. Adult GRMD dog instrumented for respiratory inductance plethysmography (RIP) and spirometry. For RIP (A), elastic bands (broad arrows) are incorporated within a spandex shirt to detect movement of the thorax and abdomen. The mesh, outer jacket protects the bands and has a pouch (narrow arrow) to hold the telemetry unit. For spirometry (B), the dog's muzzle is positioned within a rigid, plastic anesthetic mask supported by a sling (striped fabric) tied behind the ears. The mask is connected directly to a pneumotachygraph (arrow).

Fig. 2.

(A) Peak expiratory flow : peak inspiratory flow (PEF:PIF) in different age groups as measured by tidal breathing spirometry. Dogs with GRMD (▲) had higher PEF:PIF at 5–7 months of age compared with control dogs (●). The ratio of PEF:PIF increased between 2–3 and 5–7 months in GRMD dogs. (B) The increased expiratory flow in GRMD dogs persisted during expiration, as evidenced by the ratio of expiratory flow at 50% of tidal volume to the inspiratory flow at 50% of tidal volume (EF50:IF50).

Fig. 3.

(A) Peak expiratory flow : peak inspiratory flow (PEF:PIF) in three GRMD dogs measured at two (Dogs 1 and 2) or three (Dog 3) ages by tidal breathing spirometry. All three dogs showed an increase in expiratory flow between 2–3 and 5–7 months of age. (B) This progression of increased expiratory flow persisted during expiration as evidenced by the ratio of expiratory flow at 50% of tidal volume to the inspiratory flow at 50% of tidal volume (EF50:IF50).

Table 1:

Results of tidal breathing spirometry from 7 GRMD dogs and 8 control dogs at 5–7 months of age expressed as median (25th/75th percentile).

Parameter (units)	Control Dogs	GRMD Dogs	<i>p</i> Value
Respiratory Rate (breaths/min)	41.5 (28.4/45.4)	32.3 (25.2/43.9)	0.336
Time in Expiration (sec)	0.79 (0.59/0.97)	0.61 (0.54/0.70)	0.054
Time in Inspiration (sec)	0.74 (0.69/1.19)	1.07 (0.80/1.25)	0.232
Tidal Volume (mL)	202 (184/302)	189 (162/243)	0.336
Minute Volume (mL)	8270 (6650/10730)	6080 (4260/8010)	0.121
PEF (mL/sec) *	522 (383/599)	758 (598/884)	0.040
PIF (mL/sec)	364 (309/473)	303 (216/407)	0.232
EF50 (mL/sec) *	409 (285/467)	707 (509/795)	0.021
IF50 (mL/sec)	325 (278/407)	281 (191/378)	0.397
<u>Weight adjusted</u>			
Tidal Volume/kg (mL/kg)	13.4 (10.9/18.2)	16.5 (14.7/21.7)	0.072
Minute Volume/kg (mL/kg)	500 (408/605)	511 (417/702)	0.613
PEF/kg (mL/sec/kg) **	31.1 (26.6/33.7)	67.1 (46.3/79.9)	0.001
PIF/kg (mL/sec/kg)	22.7 (17.8/27.1)	29.1 (18.9/32.6)	0.397
<u>Ratios</u>			
<i>Expiratory:Inspiratory</i>			
Time Exp:Time Insp ***	0.85 (0.77/1.11)	0.55 (0.54/0.62)	<0.001
PEF:PIF ***	1.36 (1.19/1.51)	2.46 (2.31/2.59)	<0.001
EF50:IF50 ***	1.33 (0.97/1.46)	2.54 (2.31/2.67)	<0.001
<i>Peak flows:flows at 50% of TV</i>			
PEF:EF50 *	1.26 (1.16–1.34)	1.13 (1.08–1.18)	0.040
PIF:IF50	1.16 (1.08–1.19)	1.08 (1.08–1.17)	0.536

PEF=peak expiratory flow; PIF=peak inspiratory flow; EF50=expiratory flow at 50% tidal volume; IF50=inspiratory flow at 50% tidal volume; Time Exp=expiratory time; Time Insp=inspiratory time.

Significantly different between groups:

*
 $p < 0.05$;

**
 $p < 0.01$;

 $p < 0.001$.