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Short communication

Determination of Serum Lactate and Glucose in Dogs during Swimming Exercise

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Abstract

The aims of this study were to investigate the levels of lactate and glucose in dogs during swimming and to determine the maximal lactate steady state in small and large breeds of dog. Twelve healthy dogs, including small breed (n = 6) and large breed (n = 6), were the study subjects. After swimming for different periods (5, 10, 15, 20, 25 and 30 min), blood was collected from the dogs which was then used to analyze changes in lactate and glucose levels during swimming. Results showed that the mean frequency of leg movement of the small breed was 99.7 ± 15.86 times/min, significantly higher (p<0.05) than that of the large breed (71.72 ± 11.56 times/min). The levels of blood lactate and blood glucose in the two groups were not significantly different among the different exercise periods (p>0.05). However, the highest level of serum lactate was found in the small breed after 15 min of swimming. In conclusion, in the large breed of dog 30 min swimming at this intensity did not increase the serum lactate up to the maximal lactate steady state, but in the small breed the maximal lactate steady state was achieved after 15 min of swimming.

Keywords: dog, glucose, lactate, swimming

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Introduction

Nowadays, swimming is one of the most popular exercises in small-animal medicine, and has many therapeutic benefits as well. It has been reported that swimming can improve cardiovascular system function and increase muscle strength and endurance. Moreover, swimming can be beneficial by minimizing weight-bearing forces, thus reducing pain and allowing improved range of joint motion and muscle strength.

Muscle contraction needs aerobic energy at initial exercise. Upon reaching the limit of oxygen use (maximal aerobic power, or VO_2 max) another type of energy, known as anaerobic energy, will be used. When exercise is at steady stage, fatty acid metabolism is the source of aerobic energy. At a higher level of exercise, metabolism of glycogen occurs together with the metabolism of fatty acids. After the body is able to adapt, fatty acids will once again be used as the only source of energy. Continued use of muscle can cause exhaustion, or “fatigue”, due to an accumulation of lactic acid (Gobatto et al., 2001). Many researchers studied the changes in lactate and glucose levels during swimming exercise in animal models (Kooyman and Ponganis, 1994; Misumi et al., 1994; Voltarelli et al., 2002; Cunha et al., 2009) and in humans (Soultanakis et al.,2012; Sperlich et al., 2010).

The term “anaerobic threshold”, or “lactate threshold”, refers to when glycolysis occurs during exercise; this threshold can be increased by regular exercise (Wasserman, 1984; Sullivanet al.,1989). Maximal lactate steady state (MLSS) is the highest blood lactate level that can be identified as maintaining a steady-state at increased exercise (Ribeiro et al., 1990 ; Gobatto et al., 2001; Bonanni et al., 2004). It reduces the effectiveness of ATPase in muscle fibers and causes fatigue (Bonanni et al., 2004; Ribeiro et al., 1990). Thus, MLSS is an important indicator of exercise (de Keijzeret al., 1999; ; Gobatto et al., 2001; Zagatto et al., 2004; Ferasin et al., 2007;Caiet al., 2010).

However, there have been no reports on changes in serum lactate and glucose levels in dogs during swimming. The objective of this study was to investigate the levels of serum lactate and glucose in dogs during swimming and to determine the maximal lactate steady state in small and large breeds of dog.This study aimed to find the time that lactate rises to its maximum level during swimming in small and large breeds of dog, which will be beneficial for programming the duration of swimming in dogs for exercise protocol.

Materials and Methods

Animals: Twelve dogs were the subjects of this study, including 6 Pomeranians (male = 4, female = 2) and 6 Golden retrievers (male = 2, female = 4). All animals were 55.6 ± 12.05 months old and healthy with body condition scores of 4/9-6/9 (Laflamme, 1997). The Pomeranian was used as a representative of small breeds of dog, while the Golden retriever was used as a representative of large breeds of dog.

The dogs underwent a physical examination conducted by a veterinarian, blood evaluation (complete blood count and blood chemistry) and chest radiography; ventrodorsal and lateral projection were used to evaluate the heart and lungs. Complete blood count (CBC) and blood chemistry tests were conducted at the Laboratory Unit, Small Animal Hospital, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand. The blood samples were analyzed for CBC, including hematocrit and hemoglobin levels, red blood cell count (RBC), white blood cell count (WBC), and platelet count by using hematology analyser (BC-5300vet, Mindray). Two ml of serum was analyzed for blood chemicals, including alkaline phosphatase (AP), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine (Vital Flexor XL, Vital Scientific). The experimental protocol was approved (2013) by the Faculty of Veterinary Medicine and the Ethics Committee, Chiang Mai University, Thailand.

Swimming pool: The swimming pool (2.5 × 6.0 × 1.5 m, W × L × H) in this study was a chlorinated pool using calcium hypochlorite, long-lasting chlorine (J.D. Pools, Thailand). During the day, water temperature was 32 ± 3 °C with a pH range of 7.2-8.4 and a chlorine level of 0.5-2.0 ppm (Nganvongpanit et al., 2011; Nganvongpanit et al., 2014).

Pre-experimental and experimental program: All dogs were trained to swim on at least five or six occasions prior to the start of the experiment in order to prevent overly excited behavior during swimming which could affect the results (Nganvongpanit et al., 2011; Nganvongpanit et al., 2014).

During the experiment, the dogs were randomly assigned different swimming times. Each dog swam every two days for six different swimming periods, as shown in Table 1. The dogs swam without wearing swimsuits, with the researcher staying close to the dogs in the pool. Blood was collected immediately after swimming (within 30 sec) to prevent discrepancies due to time differences. To prevent the effect of exercise all dogs were not allow to play, run or do heavy exercise at least 4-6 hr before the study started.

Table 1 Experimental design: swimming times for each dog

Time	Dog number					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
1	5 min	10 min	15 min	20 min	25 min	30 min
2	30 min	5 min	10 min	15 min	20 min	25 min
3	25 min	30 min	5 min	10 min	15 min	20 min
4	20 min	25 min	30 min	5 min	10 min	15 min
5	15 min	20 min	25 min	30 min	5 min	10 min
6	10 min	15 min	20 min	25 min	30 min	5 min

Data collection: Two ml of blood was collected from the cephalic vein two times, pre- and post-swimming, to evaluate glucose and lactic acid levels. Both glucose and lactic acid evaluations were not performed under fasting condition. This study also compared glucose and lactic acid levels before and after swimming. Blood glucose was measured using blood glucose electrodes (MediSense®OptiumXceed; Abbott Diabetes Care, Doncaster, Australia). Blood lactate was analyzed using an automated high-throughput system (DiaSys Diagnostic Systems, Holzheim, Germany) at a diagnostic laboratory at MaharajNakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Thailand. Numbers of leg movement during swimming were also counted by 2 investigators using hand tally counters.

Statistical analysis: The lactate and glucose levels of the subjects were measured at 5 min intervals, from pre-swimming (0 min) to the end of the testing period (30 min), and were used to calculate relative change (data are presented as mean ± SD). Differences in mean values between the experimental groups were tested using ANOVA, followed by multiple pairwise comparisons using a *t*-test. Differences of $p<0.05$ were considered to be significant. All data were analysed using SPSS version 17.0.1 software.

Results and Discussion

All dogs enrolled in the trial had hemogram and biochemical profile results within the reference range throughout the trial (Table 2).

There was no significant difference in the mean blood lactate level in the resting stage between the healthy small breed (2.23 ± 1.23 mmol/L) and large breed (1.89 ± 0.83 mmol/L). Moreover, there was no significant difference in the mean blood glucose level between the healthy small breed (77.7 ± 10.7 mg/dL) and large breed (76.3 ± 8.36 mg/dL) in the resting stage.

The mean frequency of leg movement in the small breed was 99.7 ± 15.86 times/min and significantly higher ($p<0.05$) than that in the large breed, which was 71.72 ± 11.56 times/min.

The mean blood lactate levels in the small breed in the exercise stage after 0, 5, 10, 15, 20, 25, 30 min were 2.22, 4.29, 3.39, 5.16, 3.40, 3.83 and 3.88 mmol/L, respectively; and in the large breed were 1.89, 2.08, 1.94, 2.39, 1.98, 2.28 and 2.37 mmol/L, respectively (Table 3). There was no significant difference in the blood lactate levels between the small and large breeds of dog in the exercise stage ($p>0.05$).

Table 2 Complete blood cell count (CBC) and blood chemistry of dogs

	Reference range[a]	Small breed	Large breed
CBC			
RBC (*10^6 cells/mm^3)	5.5-8.5	7.06 ± 1.10	6.84 ± 0.53
Hemoglobin (g/dL)	12-18	14.30 ± 2.04	31.81 ± 48.98
Hematocrit (%)	37-55	42.71 ± 6.42	43.75 ± 2.82
WBC (cells/mm^3)	6,000-17,000	8,971.43 ± 2,186.10	12,687.50 ± 4,536.34
Neutrophils (%)	60-77	78.57 ± 3.46	77.88 ± 2.42
Band (%)	0-3	0	0
Eosinophils (%)	2-10	0.71 ± 1.11	0.88 ± 1.13
Lymphocytes (%)	12-30	19.57 ± 4.31	20 ± 2.14
Monocytes (%)	3-10	1.14 ± 0.38	1.25 ± 0.71
Platelet count (cells/mm^3)	200,000-500,000	373,571.43 ± 153,400.19	242,875 ± 85,467.52
Blood chemistry			
BUN (mg%)	10-22	33 ± 13.03	22.75 ± 10.39
Creatinine (mg%)	0.4-1.5	0.71 ± 0.09	0.95 ± 0.11
ALT (mg%)	5-50	79 ± 43.65	45.5 ± 24.32
AP (mg%)	20-120	12.86 ± 9.21	24 ± 18.88

RBC = red blood cells, WBC = white blood cells, BUN = blood urea nitrogen, ALT = alanine aminotransferase, AP = alkaline phosphatase

[a] Reference range (Latimer, 2011)

Table 3 Blood lactate level of small and large breeds of dog in exercise stage

Time of blood sampling during exercise	Blood lactate concentration (mmol/L)	
	Small breed	Large breed
0 min	2.22 ± 1.23	1.89 ± 0.83
5 min	4.29 ± 2.10	2.08 ± 0.86
10 min	3.39 ± 2.80	1.94 ± 0.57
15 min	5.16 ± 2.32	2.39 ± 0.98
20 min	3.40 ± 2.14	1.98 ± 1.83
25 min	3.83 ± 0.96	2.28 ± 1.22
30 min	3.88 ± 2.14	2.37 ± 1.35

Values are explanation with mean ± SD.

Table 4 Blood glucose level in exercise stage in small and large breeds of dog

Time of blood sampling during exercise	Blood glucose concentration (mg/dL)	
	Small breed	Large breed
0 min	77.69± 10.68	76.28 ± 8.36
5 min	80.17 ± 12.42	66.17 ± 10.68
10 min	94.17 ± 22.55	69.17 ± 9.95
15 min	89.17 ± 20.99	70.50 ± 6.47
20 min	90.50 ± 10.89	65.50 ± 5.39
25 min	88.50 ± 9.16	67.33 ± 8.24
30 min	86.83 ± 21.08	65.83 ± 4.92

Values are explanation with mean ± SD.

The mean blood glucose levels in the exercise stage in the small breed after 0, 5, 10, 15, 20, 25 and 30 min were 77.69, 80.17, 94.17, 89.17, 90.5, 88.5 and 86.83 mg/dL, respectively; and in the large breed were 76.28, 66.17, 69.17, 70.50, 65.50, 67.33 and 65.83 mg/dL, respectively (Table 4). There was no significant difference in the blood glucose levels between the small and large breeds of dog in the exercise stage ($p>0.05$).

This study is the first to show that swimming for 30 min had no effect on metabolism in large dogs; however, the effect was noticeable in small dogs. The serum lactate levels in the small breed gradually increased to the maximum lactate level at 15 min, and then decreased slightly until 30 min. In contrast, the serum lactate levels in the large breed continued to increase throughout the 30 min exercise period, without reaching a peak (Fig 1). It is probable that for low-intensity (submaximal) exercise in large breeds of dog, 30 min of swimming is not long enough to cause a change in the serum lactate level.

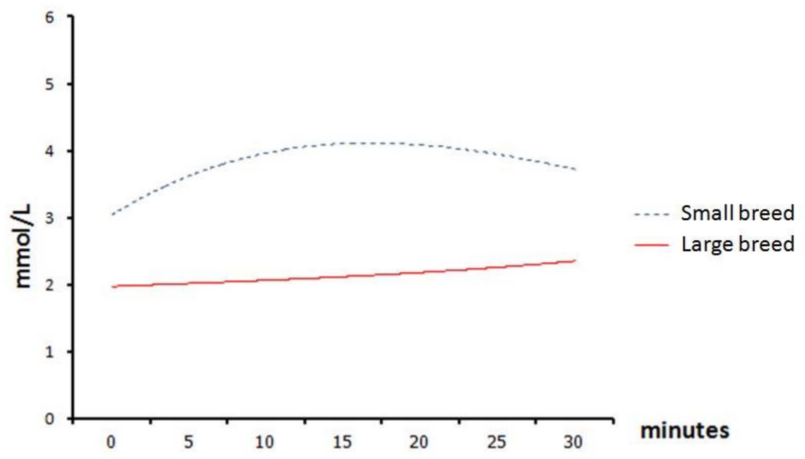

Figure 1 Blood lactate level of small and large breeds of dog during exercise

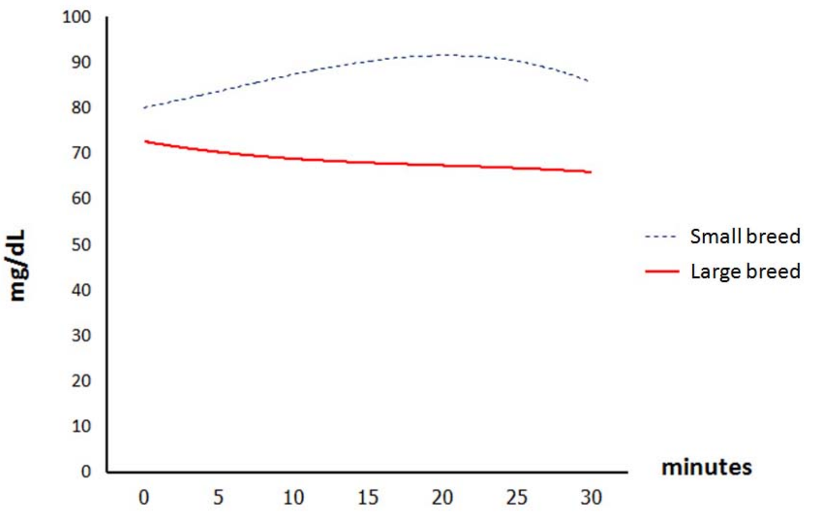

Figure 2 Blood glucose level of small and large breeds of dog during exercise

In humans, frequency of arm movement during exercise is associated with intensity of exercise, which in turn causes a difference in blood lactate level (Psycharakis et al., 2008). Some experiments in laboratory mice showed that the greater the intensity of exercise, the higher the blood lactate level (Gobatto et al., 2009). Therefore, in order to increase the blood lactate level in large breeds of dog, the intensity of exercise must be controlled. However, because of differences in the rate of leg movement between large and small breeds, serum lactate levels for the same exercise period cannot be compared.

The constant lactate level in the condition called "isocapnic buffering" causes slower lactate production, making aerobic and fat metabolism more effective (Parkhouse et al., 1985). Some studies in horses and humans have shown that at least 5 min of exercise is necessary in order to obtain representative data on lactate level in muscles (Foster et al., 1995; Lindner, 2010; Miranda et al., 2014).

Comparing the small and large breeds of dog, there were no significant differences in the blood glucose levels during the resting stage or exercise stage (Fig 2). Muscle contraction uses ATP as an energy source for metabolism; and in anaerobic energy-production systems such as glycolysis and glycogenolysis, this occurs during the initial stage of exercise, when glucose in the blood is used as the main fuel. Continuing exercise requires more energy, so the body uses another source, glycogen from the muscles and liver, in a process known as glycogenolysis, where the end product is lactic acid. In this way, glycogen, the precursor of glucose, decreases; after that, glucose is used, causing a consequent decrease in the level of blood glucose. Moreover, as noted by Ploug et al. (1990), to produce more energy during exercise, muscles must increase glucose absorption from the blood by a process called "insulin-like effect". In this experiment, the blood glucose levels in both small and large breeds of dog tended to decrease slightly. Because the experimental time was not long enough, no significant change in blood glucose level was observed.

In the large breed, swimming for 30 min did not result in increased serum lactate level, meaning that this was not a long enough period to initiate aerobic metabolism. In the small breed, the highest serum lactate level was achieved after 15 min of exercise, followed by aerobic metabolism.

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บทคัดย่อ

การตรวจสอบหาแลคเตทและกลูโคสในซีรั่มของสุนัขในขณะออกกำลังกายด้วยการว่ายน้ำ

กรกฎ งานวงศ์พาณิชย์[1,] ภาพตะวัน ดีอินทร์[1] ศจิกา สีงาม[1] เทิดศักดิ์ ญาโน[2] ปัณฑิตา เสียงดี[1] ศิริพันธุ์ คงสวัสดิ์[3]*

วัตถุประสงค์ของการศึกษานี้เพื่อตรวจสอบหาระดับของแลคเตทและกลูโคสในสุนัขขณะว่ายน้ำ และตรวจสอบหาค่าความเข้มข้นสูงสุดของแลคเตทในเลือด ในสุนัขทั้งสายพันธุ์เล็กและสายพันธุ์ใหญ่ โดยสุนัขสุขภาพดี 12 ตัว ซึ่งประกอบด้วยสุนัขสายพันธุ์เล็ก (n = 6) และสายพันธุ์ใหญ่ (n = 6) ถูกนำมาเป็นตัวอย่างในการศึกษานี้ ตัวอย่างเลือดจะถูกเก็บจากสุนัขเพื่อนำมาวิเคราะห์หาการเปลี่ยนแปลงของระดับแลคเตทและกลูโคสภายหลังจากการออกกำลังกายด้วยการว่ายน้ำโดยกำหนดเป็นช่วงระยะเวลาที่แตกต่างกันคือ 5, 10, 15, 20, 25 และ 30 นาที ผลการทดลองพบว่าค่าเฉลี่ยของความถี่ของการเคลื่อนไหวของขาในสุนัขพันธุ์เล็กซึ่งอยู่ที่ 99.7 ± 15.86 ครั้งต่อนาที มีค่าสูงกว่าอย่างมีนัยสำคัญทางสถิติที่ $p<0.05$ เมื่อเปรียบเทียบกับสุนัขพันธุ์ใหญ่ซึ่งมีค่าอยู่ที่ 71.72 ± 11.56 ครั้งต่อนาที ทั้งนี้ไม่พบความแตกต่างในทางสถิติ $p>0.05$ ของระดับแลคเตทและกลูโคสในเลือดในทั้งสุนัขพันธุ์เล็กและพันธุ์ใหญ่ในทุกช่วงระยะเวลาที่แตกต่างกันของการว่ายน้ำ อย่างไรก็ตามระดับความเข้มข้นสูงสุดของแลคเตทในเลือด ตรวจพบในสุนัขพันธุ์เล็กภายหลังจากการว่ายน้ำ 15 นาที บทสรุป ในสุนัขพันธุ์ใหญ่ถึงแม้จะว่ายน้ำเป็นระยะเวลาที่นานที่สุดถึง 30 นาที ก็ไม่สามารถที่จะเพิ่มระดับของแลคเตทให้ขึ้นถึงระดับสูงสุดของแลคเตทในเลือด ที่ตรวจพบในการทดลองนี้ได้ ในขณะที่ในสุนัขพันธุ์เล็กระดับของแลคเตทเพิ่มถึงระดับที่สูงที่สุดภายหลังจากการว่ายน้ำเพียง 15 นาที

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