THE EFFECT OF SPRINT TRAINING

ON INTRAMUSCULAR PH AND BUFFERING CAPACITY

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ABSTRACT

To determine muscle adaptations to sprint training, 9 subjects completed 15 to 20 intervals at 90 RPM, 4 days a week for 7 weeks on a bicycle ergometer adapted for one-legged pedalling. The initial intensity was set at 150 % of one-legged $\dot{V}_{O_2}$ max and was progressively increased over the 7 weeks. Needle biopsies from the vastus lateralis and blood samples from an antecubital vein were taken at rest (R), post exercise (PE) and during recovery (REC) from a 60 second one-legged maximal power test on a bicycle ergometer before and after training. Intramuscular pH and non-bicarbonate buffering capacity (BC) were determined by the homogenate technique and plasma lactate was determined enzymatically. pH at R, PE and REC was 6.92, 6.59 and 6.74 for the pre-trained state; 6.94, 6.72 and 6.75 for the post-untrained state; and, 6.90, 6.72 and 6.79 for the trained condition, respectively. Post exercise pH in both the trained (T) and untrained (UT) legs following the training period were not different but both were significantly higher than in the pre-training state (PT). BC increased from 49.5 Slykes (PT) to 57.7 Slykes in the trained leg. Blood lactate levels during recovery were higher ($p < 0.05$) for PT (8.4 mmol * l^{-1}) and T (7.8 mmol * l^{-1}) when compared to UT (6.5 mmol * l^{-1}). Peak and total power output (PO) on the 60 second one-legged power test increased significantly from the pre to post-trained state with the T leg being significantly higher than both other conditions. 1-legged

$\dot{V}_{O_2}$ max significantly increased from PT (3.36 l * min^{-1}) to UT (3.57 l * min^{-1}) and T (3.69 l * min^{-1}). 2-legged $\dot{V}_{O_2}$ max also improved significantly from 3.69 to 4.17 l * min^{-1} after training. These data suggest that sprint training enhances the ability of muscle to tolerate increased production of hydrogen ion by increasing the buffering capacity of muscle. These changes are reflected in an increased total power output in 60 seconds of intense maximal work. Furthermore, the improvements in hydrogen tolerance and BC can be accomplished in concert with improvements in $\dot{V}_{O_2}$ max.

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PREFACE

The ability to resist fatigue due to the generation of high levels of lactic acid and subsequent release of hydrogen ions is very important to the recreational and elite athlete. High intensity muscular work from thirty seconds to several minutes utilizes energy production primarily via the anaerobic lactic acid system. The rate of energy supply from this system is approximately twice that of the aerobic system, however, the capacity is much less due to the buildup of lactic acid (Fox & Mathews, 1981). The rate of production of lactic acid increases with the intensity of exercise. The intramuscular accumulation of hydrogen ions (H^+) from the dissociation of lactic acid to lactate results in an inhibition of the glycolytic pathway and ultimately, fatigue. Thus, the ability to carry on muscular work at a high intensity depends on the ability of the muscle to remove or tolerate the high levels of lactate and/or H^+.

During acute bouts of exercise muscle lactate levels can elevate to 40 mmol * kg^{-1} wet weight (Shephard, 1984). In association with the lactate, an equimolar amount of hydrogen ion is produced which has shown to result in a decreased intracellular pH from 7.04 to 6.41 after maximal exercise (Hultman & Sahlin, 1980; Hermansen & Osnes, 1972). The increased concentration of hydrogen ion (and decreased pH) results in at least the following: an inhibition of phosphofructokinase, the rate limiting step in glycolysis; a hyperpolarized membrane potential; competition of hydrogen ion with calcium ions at troponin;

and, an inhibition of calcium ions released from the sarcoplasmic reticulum (Wenger & Reed, 1976). The combined effect is a reduction in glycolytic energy supply and subsequent force production in the muscle.

The ability of the muscle to tolerate high levels of H^+ and resist the change in pH has been termed buffering capacity (van Slyke, 1922) and becomes an important factor in the performance of high intensity exercise. This tolerance has been observed in trained athletes (Hermansen & Vaage, 1977; Karlsson, 1971) and is probably due to physiochemical buffering; consumption or production of nonvolatile acids; and, transmembrane fluxes of H^+ or bicarbonate (Siesjo & Messeter, 1971). Anaerobically trained athletes have shown an enhanced buffering capacity when compared with endurance trained or untrained individuals (Parkhouse et al., 1985; Sahlin and Henriksson, 1984). Although this suggests that the type of training program may influence buffering capacity there is no research that links anaerobic or sprint training programs to enhanced intracellular buffering capacities.

Thus, the purpose of this study is to investigate the effect sprint training on the tolerance of skeletal muscle to high concentrations of hydrogen ion. The following are the specific purposes:

1. To determine the intramuscular pH and blood lactate level before and after acute exercise.
2. To determine the effect of sprint training on intramuscular pH, blood lactate and buffering capacity.
3. To determine whether sprint training enhances power output on a sixty second maximal power test.
4. To determine the influence of sprint training on aerobic power.

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A special thank you to my fellow graduate students and staff of the fitness and testing centre for their continuous support over the last two years. Finally, I thank my wife, Tracey, for her love and support throughout my graduate program.

DEDICATION

This thesis is dedicated to my mother and to the memory of my late father, who placed a high value on my continued education.

THE EFFECT OF SPRINT TRAINING ON INTRAMUSCULAR PH AND BUFFERING CAPACITY

Introduction

The ability of skeletal muscle to function at a high intensity is partly dependent on the acid-base status of the intra and extracellular environment. This balance is affected during short term maximal exercise in which glycolytic breakdown of carbohydrate results in the production of lactic acid (Fox and Mathews, 1981). Sahlin (1978) has shown that upon generation, lactic acid dissociates rapidly to lactate and hydrogen ion (H^+) The increased concentration of H^+ and subsequent drop in pH are directly related to tension decline and slowing of relaxation rate in skeletal muscle (Sahlin et al., 1981). Therefore, the ability of skeletal muscle to tolerate intramuscular pH and lactate changes becomes an important factor in the function of muscle exposed to these conditions.

The response of sprint-trained muscle to exercise is different from endurance or untrained muscle (Medbo & Sejersted, 1985; Cheetham, Williams & Lakomy, 1985; Costill et al., 1983). Sprint-trained athletes work at higher rates and attain higher blood lactates than endurance trained athletes when compared on similar performance tests (Parkhouse et al., 1985; Medbo & Sejersted, 1985). Costill et al., (1983) observed lower muscle pH in sprinters when compared to endurance trained individuals after a maximal treadmill sprint run. Anaerobically trained

athletes attained higher muscle pH levels after maximal isometric contraction than sedentary subjects (Sahlin & Henriksson, 1984). Buffering capacity of muscle was observed to be higher in anaerobically trained individuals (Sahlin & Henriksson, 1984; Parkhouse et al., 1985) and in high glycolytic muscle of various animals (Castellini & Somero 1981). Although these differences indicate that anaerobically trained individuals have an enhanced ability to work under high intensity exercise, there is little research linking the improvements in acid-base balance with sprint training. Therefore, the purpose of this study is to analyze intramuscular pH and buffering capacity after a sprint training program. Changes in maximal power output, blood lactate and maximum oxygen consumption will also be assessed.

Methods

Subjects

Eight male and one female from the University of Victoria signed informed consent and volunteered to act as subjects. Physical characteristics are shown in Table 1. All subjects were familiarized with testing and training procedures prior to the training program.

Table 1

Physical characteristics of the subjects

Subject	Age (year)	Height (cm)	Weight (kg)	Sum of Skinfolds * (mm)
DA	24	180.0	86.0	80.9
BB	27	172.7	65.2	56.5
GB	29	164.8	73.0	58.0
RC	30	179.5	77.8	62.1
TD **	28	165.8	62.0	73.5
JE	20	180.2	81.4	47.2
CP	26	179.5	83.0	49.4
KP	30	167.5	62.6	33.9
GS	22	183.3	82.8	80.6
$\bar{x}$	26.2	171.1	74.9	60.2
SD	3.4	7.2	9.5	15.9

* sum of six skinfolds = tricep + bicep + subscapular + suprailiac + front thigh + medial calf.

** female

Testing Procedure

The testing and training procedure is presented in Figure 1. All subjects were asked to refrain from any physical activity one day prior to testing. Anthropometric measures included height, weight and the sum of six skinfolds (tricep, bicep, subscapular, suprailiac, front thigh and medial calf). One and two-legged aerobic power $\dot{V}_{O_2}$ max and heart rate (HR) were determined on consecutive days during an incremental bicycle ergometer test to fatigue as described by Neary and Wenger (1985). Respiratory gases were collected and analyzed every 30 seconds on a Beckman Metabolic Measurement Cart. Heart rate was recorded every minute throughout the test and every 30 seconds for 3 minutes during recovery on a electrocardiogram (Cambridge VF4).

On the following day, subjects completed a one-legged 60 second maximal anaerobic power test on a bicycle ergometer adapted for one-legged pedalling. Resistance was set at 0.035 kg * kg body weight^{-1} Peak 5 second and total 60 second power output (PO) over the 60 second test were determined from pedal revolutions. All one-legged testing in the pretest situation was conducted on the leg chosen to train.

Muscle samples were obtained by needle biopsy (Bergstrom, 1962) adapted for suction (Evans et al., 1982) from the medial aspect of the vastus lateralis muscle prior to, immediately after and during recovery from exercise. Biopsy 1 was taken at rest on the leg opposite to the leg performing the 60 second power test. Biopsy 2 and 3 were taken approximately 1 minute and after four minutes of recovery from the power test, respectively. Blood samples from an antecubital vein were taken to coincide with the muscle biopsies.

Figure 1: The Testing and Training Schedule for All Subjects.

Leg Involved	Testing Days <- 1 2 3 ->	<- 7 Weeks ->	Testing Days 1 2 3 4 5
right	Pre-training	<- Sprint Training ->	Post-training
left		<- No Training ->	

Pre Test:

Day 1 - Body weight, height, skinfolds, $\dot{V}_{O_2}$ max (two-legged) and heart rate.

Day 2 - One-legged $\dot{V}_{O_2}$ max and heart rate on the leg to be trained.

Day 3 - A muscle biopsy and blood sample at rest, post exercise and after 3 minutes of recovery on the leg to be trained

Post Test:

Day 1 - same as Day 1 in pre test.

Day 2 - same as Day 2 in pre test using the trained leg.

Day 3 - same as Day 2 in pre test using the untrained leg.

Day 4 - same as Day 3 in pre test using the trained leg.

Day 5 - same as Day 3 in pre test using the untrained leg.

After training, both the trained and untrained leg were biopsied as described above except that the resting biopsy was taken on the same leg undergoing the 60 second power test on that day.

Biochemical Analyses

Muscle tissue samples were removed from the biopsy needle and frozen in liquid nitrogen. The samples were wrapped in labelled tinfoil and stored at -80 degrees Celcius. Tissue samples were analyzed for pH utilizing the homogenate technique as described by Costill et al. (1982). Subsequently, buffering capacity was determined according to the method of Albers & Vaupel (1981). The homogenates were adjusted to pH 7.00 + 0.02 with 0.1 N NaOH and repeatedly titrated (3 times) to pH 6.00 + 0.02 with 0.01 N HCl. Nonbicarbonate buffering capacity was defined as umoles of HCl required to titrate 1 gram of muscle, 1 pH unit (umol HCl * g^{-1} * pH^{-1}). This measure has been termed a Slyke (van Slyke, 1922).

Venous blood samples were deproteinized in 4% perchloric acid, centrifuged and analyzed spectrophotometrically according to Sigma method 826-UV (Sigma Chemical Company, 1981) for blood lactate.

Training Program

The one-legged sprint training program consisted of 15 to 20 intervals of 20 second duration at a work to rest ratio of 1:3. Training was 4 times a week for seven weeks. Intensity was set at 150% of the initial PO which elicited one-legged $\dot{V}_{O_2}$ max and was increased throughout the training regimen. Pedal revolutions were set at 90 RPM. Subjects were required to complete at least 15

intervals and when 20 intervals were achieved on two consecutive sessions, resistance was increased by 10%.

Heart rate was monitored throughout the training sessions using Benchmark Quantum XL portable monitors. All testing and training was conducted on a Monarch bicycle ergometer (Model 876) which was secured to the floor. The ergometers were adapted for one-legged pedalling by removing one pedal and using a toe clip and heel strap to secure the foot. The non-training leg remained stationary on the bicycle ergometer crossbar.

Paired Student t-tests were utilized to determine statistical significance between the pre and post-trained states as well as between the post-trained and untrained conditions.

Results

Intramuscular pH was not altered with training at rest (R) or recovery (REC). pH after exercise was significantly increased in both the untrained (UT) and trained (T) leg after the training program when compared to the pre-trained leg (PT). Blood lactate at R and post exercise (PE) was not significantly altered with training, however, REC blood lactate was significantly higher in T when compared to UT. Buffering capacity (BC) significantly improved with training (Table 2).

Peak PO (5 second) on the 60 second maximal power test showed significant improvement in the T leg only. Total PO showed significant increases in both the UT and T conditions (Table 3).

Absolute ($l * min^{-1}$) and relative ($ml * kg^{-1} * min^{-1}$) two-legged $\dot{V}_{O_2}$ max significantly increased with training. However, a reduction in body weight was

Table 2

Pre-trained (PT), post-untrained (UT) and post-trained (T) measures of pH and blood lactate at rest (R), post exercise (PE) and recovery (REC) and pre-post trained measures of buffering capacity.

	pH			Blood Lactate (mmol× l^{-1})			Buffering Capacity
Condition	R	PE	REC	R	PE	REC	(Slykes)
PT	6.92	6.59	6.74	0.9	5.8	8.4	49.5
	(.04)	(.02)	(.03)	(0.2)	(0.8)	(0.2)	(1.1)
UT	6.94	6.72 a	6.75	1.1	5.7	6.5 b	52.9
	(.07)	(.02)	(.02)	(0.1)	(0.5)	(0.8)	(1.8)
T	6.90	6.72 a	6.79	1.0	6.2	7.8	57.7 b
	(.04)	(.07)	(.02)	(0.5)	(0.6)	(0.6)	(1.8)

a = significantly different from pre-trained condition, $p<.05$.

b = significantly different from both other conditions, $p<.05$.

Table 3

Peak 5 second power output (PO) and total PO on a 60 second maximal power test before and after training.

Condition	Peak 5 sec. PO (Watts)	Total 60 sec. PO (Watts)
Pre-trained	368 ± 21	251 ± 11
Post-untrained	357 ± 20	271 ± 13 a
Post-trained	403 ± 20	296 ± 16 b

a = significantly different from pre-trained condition, $p<.05$.

b = significantly different from both other conditions, $p<.05$.

also observed which may have influenced relative $\dot{V}_{O_2}$ max increases. One-legged $\dot{V}_{O_2}$ max was significantly enhanced in both UT and T but no significant difference was observed between UT and T for absolute $\dot{V}_{O_2}$ max . However, significance was observed between UT and T on relative $\dot{V}_{O_2}$ max scores. Two-legged power output (PO) at $\dot{V}_{O_2}$ max was significantly enhanced after training while one-legged PO significantly increased between pre and post training but not between UT and T. Heart rate at $\dot{V}_{O_2}$ max was not altered with training on either one or two-legged tests (Table 4).

Table 4

Mean one and two-legged aerobic power, power output (PO) and maximum heart rate (HRmax) in the pre-trained (PT), post-untrained (UT) and post-trained (T) conditions.

Condition		$\dot{V}_{O_2}$ max ($l \times min^{-1}$)	$\dot{V}_{O_2}$ max ($ml \times kg^{-1} \times min^{-1}$)	PO at $\dot{V}_{O_2}$ max (Watts)	HRmax ($b \times min^{-1}$)
1-legged	PT	3.36 (0.18)	45.4 (2.4)	177 (10)	187 (2)
	UT	3.57 a (0.19)	48.8 a (2.6)	207 a (11)	189 (4)
	T	3.69 a (0.20)	50.1 a (2.0)	226 a (9)	188 (3)
2-legged	PT	3.96 (0.20)	52.9 (2.1)	308 (10)	197 (3)
	T	4.17 a (0.21)	56.9 a (2.2)	336 a (16)	192 (2)

a = significantly different from pre-trained condition, $p<.05$.

Discussion

The homogenate technique is one of the oldest techniques for measuring intramuscular pH (Costill et al., 1982) and the criticisms of it are well documented (Roos & Boron, 1981). pH utilizing this technique has been reported to range from 6.92 to 7.10 at rest and 6.41 to 6.87 after high intensity exercise (Costill et al., 1983; Sahlin et al., 1978; Hermansen & Osnes, 1972). These variations may be due to methodological differences and handling of the tissue. Resting, post exercise and recovery pH values in this study fall within these ranges. The slightly lower pH at rest in the present study may have resulted from the time delay in extraction and freezing. Information regarding pH changes during recovery are few. However, Sahlin et al. (1978) reported muscle pH values of 6.64 and 6.86 at 1 and 8 minutes of recovery. The four minute recovery pH in the present study was 6.74, 6.75 and 6.79 for the PT, UT and T conditions respectively which were within the range reported by Sahlin et al. (1978).

One advantage of using the homogenate technique is that buffering capacity measures can be conducted with the same sample used for pH determination. Non-bicarbonate buffering capacity has been reported in a wide variety of animal tissues (Castellini & Somero, 1981; Albers and Vaupel, 1981), however, no studies to date have demonstrated this measure in man. Sahlin and Henriksson (1984) calculated buffering capacity to be 58.8 and 49.8 Slykes in anaerobically trained and untrained subjects, respectively. These values are compatable with the results found for non-bicarbonate buffering capacity in the present study. Buffering capacity in deproteinized muscle has shown a similar trend with sprinters and rowers being significantly higher than marathoners or untrained

individuals (Parkhouse et al., 1985). The notion that enhanced buffering capacity is partially due to the training regimen in these athletes is supported by the present results.

Blood lactate response to maximal one-legged exercise is difficult to compare due to differences in mode, length of the test, and differences in sampling times. However, the blood lactate levels reported in this study are slightly lower than other reports of maximal one-legged exercise (Stamford et al., 1978; Saltin et al., 1976). The present study showed a greater blood lactate level four minutes after exercise in the trained leg when compared with the untrained leg. Saltin et al. (1976) observed a similar response in one-legged sprint training. This finding suggests that sprint training may enhance the ability of muscle to generate lactate and remove it from muscle.

The major adaptation in muscle pH occurred in the post exercise condition where pH levels were significantly higher after training. The significant increases in buffering capacity, blood lactate and power output on the 60 second power test suggest that the increased H^+ in the trained leg was consumed by buffering processes. This supports studies showing significantly higher buffering capacity in anaerobically trained athletes (Parkhouse et al., 1985; Sahlin & Henriksson, 1985). pH in the untrained leg was also significantly higher after exercise, but the significantly lower BC, blood lactate and 60 second PO suggests that the buffering effect did not occur to the same extent.

The significant improvements in one and two-legged $\dot{V}_{O_2}$ max and PO at $\dot{V}_{O_2}$ max illustrate that a sufficient demand was placed on the cardiovascular system with the sprint training intervals which elicited changes in the oxygen transport

system. This finding is consistent with Saltin et al. (1976) who also observed improvements in $\dot{V}_{O_2}$ max with high intensity one-legged sprint training. Heart rate after the work intervals ranged from 85 to 90 % of one-legged HRmax indicating that a sufficient demand was placed on the cardiovascular system which partly contributed to the increases.

Although some marathoners have been shown to have lower BC and similar $\dot{V}_{O_2}$ max when compared with sprint trained individuals (Parkhouse et al., 1985), these data suggest that one is not achieved at the expense of the other but rather the two can be increased in concert. The high volume training programs typically followed by marathoners which enhance $\dot{V}_{O_2}$ max may not provide a sufficient intensity to evoke a change in BC.

Improvements observed in the untrained leg at the end of the training program may have been the result of central circulation adaptation or some training was received by the untrained limb. The central phenomena suggests that one-legged training causes improvement in the central circulation which is transferred to non-trained muscles. This central effect with one-legged training has been shown previously (Saltin et al., 1976) and supports the present data. Secondly, it was observed that the untrained leg was not in a resting state during the work interval but was active in body stabilization and assisted the trained leg to overcome the initial resistance at the start of the work interval. This combined effect may have caused the training benefits acquired by the untrained leg.

This study suggests that sprint training intervals of 1:3 (20 sec: 60 sec) work to rest at 150% of $\dot{V}_{O_2}$ max enables skeletal muscle to tolerate increased H^+ production during high intensity exercise by enhancing the buffering capacity. This

was observed in the post trained state in which pH measures were significantly higher after maximal exercise when compared to the pre-untrained condition. Furthermore, buffering capacity due to nonbicarbonate processes was significantly enhanced. Finally, this study revealed that high intensity short duration interval work can significantly increase cardiovascular fitness as evidenced by the improvements in aerobic power. These increases in $\dot{V}_{O_2}$ max and BC suggest that the two can be improved in concert.

SUMMARY

1. Intramuscular pH was significantly higher in the untrained and trained leg after the training period when compared to the pretrained condition.
2. Buffering capacity was significantly increased with training.
3. Blood lactate levels during recovery were significantly higher in the trained leg when compared to the untrained leg but were not significantly different from the pre-trained state.
4. Peak and total power output on a sixty second power test was significantly enhanced in the trained state and was significantly higher in the post-trained leg when compared to the post-untrained leg.
5. One and two-legged $\dot{V}_{O_2}$ max and PO at $\dot{V}_{O_2}$ max was significantly enhanced with training.
6. Sprint training was able to enhance the ability of skeletal muscle to generate higher power output by tolerating increased hydrogen ion concentration by increasing buffering capacity. The combined ability of enhancing buffering capacity and aerobic power with sprint training suggest that the two may be improved in concert.

FURTHER RESEARCH

1. Further research is suggested to standardize the homogenate technique of measuring nonbicarbonate buffering capacity as methodology differences exist in the literature.
2. It is recommended that different training programs (i.e. aerobic power or strength training) be implemented to determine which will have the greatest effect on the tolerance of increased hydrogen ion concentration.
3. Future research in measuring the intracellular components of buffering capacity and their individual capacities would enable investigators to isolate which of these buffering processes adapt with training.
4. The measurement of muscle lactate corresponding with blood lactate levels during exercise is recommended in future research to provide a better understanding of the influence of training on the acid-base status of muscle.

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Appendix A

INFORMED CONSENT FOR RESEARCH ON THE EFFECTS OF SPRINT TRAINING ON BUFFERING CAPACITY, LACTATE AND PH OF SKELETAL MUSCLE

Testing Procedure

During initial testing you will be asked to perform a one and two-legged maximal aerobic power test and a 60 second maximal anaerobic power test on the bicycle ergometer. Heart rate and oxygen consumption will be monitered utilizing a ECG and the Beckman Metabolic Cart. Height, weight and skinfolds will also be determined. A small section of muscle will be taken from your thigh (vastus lateralis muscle) using the Bergstrom needle biopsy procedure adapted for suction at rest and twice following the 60 second bike test on one leg. Blood sampling will coincide with the biopsies.

The sprint training program will begin 1 week later and will require high power sprint training consisting of repeated intervals of 20 second maximal sprints followed by 60 second rest periods. The number of sessions will be 4 per week for seven weeks. After completion of the sprint training program, the same testing procedures that occurred in the initial testing session will be repeated except that both legs will be tested and biopsied to determine the training effect.

The muscle biopsy consists of a 5 mm incision in the skin and muscle fascia under local anaesthetic. A hollow "needle" is inserted approximately 1 cm into your muscle. The needle will extract a small amount of tissue (approximately 40-80 mg) which will be analyzed for pH, lactate and buffering capacity. There will be little or no discomfort associated with the procedure, however, you will feel a sensation of pressure. A 1 to 2 ml sample of blood will be taken via venipuncture from the anterior cubital area of the arm for determination of plasma lactate.

The needle biopsy procedure is to be performed by a skilled physician. Blood samples will be conducted by a qualified technician. Both procedures are considered safe and have little risk of complication. Several hours past sampling you may experience minor bruising at the biopsy site.

I have read the above and agree to participate in this research project at my own risk. I am nineteen years of age or older and engage in exercise of a similar level as a part of my regular regimen. I realize that I may expect a thorough explanation and/or demonstration of any procedures and that I may terminate participation at any time in any or all procedures of my own volition. I will also be assured anonymity of all results gathered in this research.

Having voluntarily assumed participation and the risks thereof, in the project, I hereby disclaim and release the University of Victoria, its agents, servants or employees, including all personnel involved in the research project, from any and all liability that might otherwise arise as a result of my participation as a research subject in this study.

Name: _____

Date: _____

Address: _____

Phone:

_____

Signature:

_____

Appendix B

REVIEW OF LITERATURE

For a muscle to shorten and work, chemical energy is required. This energy is produced when the molecule adenosine triphosphate (ATP) is hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate (Pi). However, the concentration of ATP in skeletal muscle is quite low (4-6 mM * kg^{-1}) and must be continually resynthesized for muscle contraction to continue. This is accomplished by three distinctly different but integrated systems (Houston, 1978)

The immediate source of energy for the synthesis of ATP is liberated through the breakdown of creatine phosphate (CP) to creatine (Cr) and inorganic phosphate (Pi) in the presence of the enzyme, creatine kinase. This system is most important during the inital stages of muscular work and is able to regenerate ATP at a rapid rate. However, the capacity of the CP system is limited, lasting for only 10 to 30 seconds of intense muscular work (Fox & Mathews, 1981).

Further regeneration of ATP requires the breakdown of carbohydrates and the oxidation of fatty acids. In the presence of oxygen, glucose and free fatty acids are oxidized utilizing the electron transport system and citric acid cycle, respectively. The net result is the formation of carbon dioxide, water and energy. These two pathways form the aerobic system and can produce energy to resynthesize ATP for many hours of work, however, the rate of resynthesis is relatively slow (Houston, 1978).

In the absence of oxygen, pyruvate, derived from the glycolytic breakdown of stored glycogen (or glucose), is reduced to produce lactic acid which further dissociates to lactate and hydrogen ion. This process is termed anaerobic glycolysis or the lactic acid system and is able to supply ATP rapidly but is limited to 2 or 3 minutes of intense work due to the accumulation of hydrogen ions (Fox & Mathews, 1981).

Since the introduction of the Duchenne biopsy needle by Bergstrom (1962) it became possible to study the chemical changes occurring in muscle of exercising man (Harris et al., 1977). The biopsy needle has proved of value in studies of intracellular lactate generation (Chirtel et al., 1984; Jacobs et al., 1983; Harris et al., 1977), intracellular pH (Costill et al., 1982; Sahlin et al., 1979; Steinhagen et al., 1976) and buffering capacity of skeletel muscle (Parkhouse et al., 1985; Sahlin & Henriksson, 1984; Castellini & Somero, 1981).

Lactic Acid Production

At rest and during muscular exercise, certain concentrations of lactate have been observed. According to du Bois-Reymond (1877), Berzelius reported the first observation of lactate in the muscles of exhausted game in 1841. However, an adequate explanation of the production of lactic acid could not be given. In 1891, Araki demonstrated an increase in lactate concentration in blood and urine when dogs, rabbits and hens were subjected to hypoxia. Orshov (1931) found that a certain amount of lactate (2 - 8 mmoles * kg^{-1} wet weight) was present in the muscles of the resting animal, supposedly well supplied with oxygen. In the same experiment the concentrations were shown to rise in the exercising muscles of intact animals to values between 20 and 30 mmoles * kg^{-1} wet weight. Subsequently, Lundsgaard (1932) coined the term "lactacid anaerobic energy production" which referred to that portion of the total energy output occurring through the incomplete breakdown of carbohydrates to lactic acid (Karlsson, 1971).

It was first thought that lactate was formed only when oxygen delivery was inadequate, but it is now clear that it may be produced at all levels of work except perhaps very light exercise continued for a long time (Jones, 1980). In steady state exercise a constant plasma lactate concentration indicates a balance between its production and consumption in muscle and its uptake from blood by various tissues such as other muscle, heart and liver (Fox & Mathews, 1981; Jones, 1980). However, as the intensity of muscular exercise increases so does the production of lactic acid (Hermansen, 1971; Karlsson, 1971). This results in at least the following: immobilization of free fatty acids (Boyd et al., 1974; Issekutz et al., 1966); a decrease in muscle and blood pH (Jones, 1980; Sahlin, 1978); and an inhibition of glycolytic enzymes such as lactate dehydrogenase (Karlsson et al., 1974) and phosphofructokinase (Edgerton et al., 1973; Danforth, 1965) which is the rate limiting enzyme in glycolysis (Wenger & Reed, 1976).

Lactate in mammalian skeletal muscle has been measured during dynamic (Chirtel et al., 1984; Jacobs et al., 1983; Jorfeldt et al., 1978) and sustained isometric contractions (Sahlin & Henriksson, 1984; Sahlin et al., 1981; Tesch & Karlsson, 1977). The highest lactate levels in the muscle have been observed at exhaustion and range from 18 - 40 mmol * kg^{-1} wet weight (Shephard, 1984). Differences in the accumulation of lactate in the muscle are due to the type of contraction, intensity of exercise and the degree of training. Sahlin and Henriksson (1984) suggest that muscle lactate levels are higher after isometric when compared with dynamic exercise due to the circulatory restriction since maximal lactate concentrations are observed at 40-50% of maximal voluntary contraction (Tesch & Karlsson, 1977; Karlsson et al., 1975).

Training can alter the production of lactic acid in the muscle. Karlsson (1971) showed the mean muscle lactate content following maximal dynamic exercise to be significantly higher in trained than untrained subjects. Also, given the same relative exercise intensity, lactate concentration has been shown to be lower in trained subjects than untrained (Hermansen, 1971; Karlsson, 1971).

Lactate Efflux

During and after muscular exercise, the intracellular lactate is either oxidized or gradually released from muscle to blood (Dodd et al., 1984; Seo, 1984; Jorfeldt et al., 1978). Marked differences between skeletal muscle and blood lactate concentrations during heavy exercise have been observed demonstrating a concentration gradient from the muscle to the blood (Karlsson, 1971).

Hermansen and Vaage (1977) found that the disappearance of lactate from muscle was rapid (mean = 0.74 mmol * min^{-1} * kg^{-1} wet weight corrected for changes in muscle water content) after maximal exercise and that the disappearance from blood is slower. Jorfeldt et al. (1978) also revealed a linear relationship between the release of lactate from the muscle to the blood of approximately 4 - 5 mmol * min^{-1} followed by a leveling off after the 4th minute of recovery.

The rate of lactic acid removal from muscle and blood has been shown to be approximately 25 minutes of rest-recovery to remove half of the accumulation (Karlsson & Saltin, 1970). The speed of removal can be enhanced by performing an active exercise recovery of low intensity (Dodd et al., 1984; Weltman et al., 1979; Belcastro & Bonen, 1975).

Hermansen and Vaage (1977) suggest that only 10% of the generated lactate in muscle is actually released into the circulation. They suggest the major fraction (90%) must be metabolized in the cell. This was supported by Mazzeo et al. (1986) who concluded that lactate turnover was directly related to the metabolic rate, oxidation is the major fate of lactate during exercise and that blood lactate was not an accurate indicator of lactate disposal. The fate of lactate has been summarized as follows (Fox & Mathews, 1981, p. 47):

1. A small amount is excreted in the urine and sweat.
2. A minor fraction is converted to glucose and/or glycogen.
3. A small amount is converted to protein.
4. The majority is oxidized/converted to CO_2 and H_2O, that is,it can be used as a metabolic fuel for the oxygen system, mostly by skeletal muscle, but also by the heart, brain, liver and kidney.

Intramuscular pH

According to the Bronsted theory, acids are substances which liberate hydrogen ions and bases are substances which react with hydrogen ions (Mortimer, 1983; Hultman & Sahlin, 1980). For example, water (H_2O) reacts with hydrogen protons (H^+) to form the hydronium ion (H_3O^+). In this case water is a base and the hydronium ion is an acid (Rafelson & Binkley, 1965). In future discussion, hydrogen ion (H^+) will be used but it will be understood that it actually exists in aqueous solution as the hydronium ion.

The amount of hydrogen ions in a solution is measured as the activity of the hydrogen ion. In dilute solutions present in biological systems, the activity of the

hydrogen ion can usually be considered equal to the concentration of the hydrogen ion. The amount of hydrogen ions in a solution can also be expressed as a pH value where pH equals the negative logarithm of the hydrogen ion concentration. The advantage of the pH scale is that a certain change in pH will always result in the same relative change of the hydrogen ion constant (Hultman & Sahlin, 1980).

During intense dynamic and static exercise muscle lactate content increases about 30-fold (Sahlin, 1983). In addition to lactate, other acidic compounds such as glucose 6-P, glycerol 1-P, pyruvate, citrate and malate will accumulate. However, lactic acid contributes to more than 85% of the liberated hydrogen ions (Sahlin, 1983; Jones, 1980). At physiological pH, an equimolar amount of hydrogen ion is produced in association with lactate (Hultman & Sahlin,1980; Sahlin, 1978; Sahlin et al., 1976).

The measurement of intracellular pH is controversial, first because of the difficulties in measuring muscle pH and, second, because pH in the cytosol of the muscle cell may differ from that in the mitochondria or other organelles due to the compartmentalization of key reactions and thus of H^+ (Jones, 1980). At present, four different types of methods are used to measure intracellular pH: indicators, DMO (5, 5-dimethyl-2, 4-oxazolidinedione) distribution, nuclear magnetic resonance, and pH-sensitive microelectrodes (Thomas, 1984). A complete review of the methods for measuring muscle pH and their inherent strengths and weaknesses has been presented by Roos and Boron (1981).

One of the oldest methods is the measurement of pH in a muscle homogenate (Costill et al., 1982). However, there has been little consistency in reported resting and post exercise pH levels. This may be due to variations in the handling

and preparation of the homogenate (Costill et al., 1982). A major criticism of the homogenate technique for measuring muscle pH is based on the continued breakdown of glycogen and the acidification of the mixture during the period of handling. To prevent glycolysis and a fall in pH within the homogenate, Sahlin (1978) added 5mM iodoacetic acid (IAA) to the KCl-NaCl medium and Costill et al. (1982) showed that this halted glycolysis. They also suggested that the homogenate cocktail be 10 ml * g^{-1} wet weight as this dilution did not clog the microelectrode and gave more stable readings than dilutions of 1:5 or 1:20. Furthermore, Costill et al. (1982) suggested that homogenate derived pH values would be higher than found in vivo due to CO_2 liberation during grinding and exposure to ambient air.

The measurement of muscle pH utilizing the homogenate technique represents the pH of the mixture of muscle fibers as well as the fluids of the intra- and extracellular compartments. Sahlin (1978) has estimated that the extracellular fluid in the homogenate will increase the measured pH in the muscle by only 0.03 units. This estimate assumes that intracellular water in the sample is 89% of the total muscle H_2O, that the extracellular volume and pH are 11% of total muscle water and 7.4, respectively, and the buffering capacities of the extracellular fluids are equal. Thus, the term "intramuscular" is preferred to "intracellular" to describe pH utilizing the homogenate technique.

Previous research has shown resting intramuscular pH of human skeletal muscle to be in the range of 6.98 to 7.08 (Costill et al., 1983; Sahlin, 1983; Costill et al., 1982). A decrease in muscle pH varies according to the intensity and type of contraction and tends to be slightly higher after dynamic bicycle exercise than

after isometric contraction. In comparing intramuscular pH after exhaustive exercise, Sahlin (1983) demonstrated that pH was 6.60 and 6.56 for dynamic and isometric exercise, respectively. The higher pH after dynamic exercise may be due to an exchange of H^+ with the blood whereas during isometric contraction little or no circulation occurs in the contracting muscle (Sahlin, 1983). However, Hermansen and Osnes (1972) observed the pH in muscle homogenates to reach a low of 6.41 after maximal bicycle exercise.

The result of a decreased muscle and blood pH are a reduction or inhibition of phosphofructokinase (PFK); a hyperpolarized membrane potential; a competition of H^+ with calcium (Ca^{++}) ions; and an inhibition of Ca^{++} release from the sarcoplasmic reticulum (Wenger & Reed, 1976). These combined effects cause a reduction in the glycolytic energy production and subsequent force production of the muscle.

Buffering Capacity

As has been previously mentioned, hydrogen ions are released within the muscle cell during exhaustive exercise. If these hydrogen ions are added to an unbuffered solution, the concentration of H^+ would amount to approximately 35 $mmol*l^{-1}$ and pH would decrease to a low of 1.5 (Hultman & Sahlin, 1980). However, the lowest muscle pH reported was 6.41 after exhaustive exercise (Hermansen & Osnes, 1972). The concentration of the released H^+ must be taken up by different buffering processes. The extent of pH decrease after addition of H^+ is determined by the buffering capacity (Hultman & Sahlin, 1980).

The buffer capacity or buffer value of a solution was defined by van Slyke (1922) as the amount of free H^+ or OH^- to be added in order to produce a change in pH of 1 unit. Woodbury (1965) suggested that buffering capacity be measured in umoles * $liter^{-1}$ * pH^{-1} and further designated the unit of measure as a Slyke.

According to Siesjo and Messeter (1971), buffering capacity in the muscle cell can be divided into:

1. Physico-chemical buffering.
2. Consumption or production of nonvolatile acids.
3. Transmembrane fluxes of H^+ or bicarbonate (HCO_3^-).

Physico-chemical buffering comprises the buffering which occurs in the cell merely as a consequence of association of H^+ with weak bases. The amount of hydrogen ions taken up by different bases can be calculated from the Henderson-Hasselbach equation provided the pKa values of all the weak acids and their conjugate bases are between 6.0 and 7.5 in the cell (Hultman & Sahlin, 1980; Sahlin, 1978). Rafelson and Binkley (1965) define the pKa as the negative logarithm of the ionization constant (Ka) and is the pH at which the concentration of acid is equal to the concentration of the conjugate base.

The consumption or production of acids or bases comprises the buffering which occurs as a consequence of enzymatic activity. This is also referred to as the metabolic buffering processes (Hultman & Sahlin, 1980) and is dependent on metabolic regulation and calculated values. This is because the metabolic buffering processes cannot be estimated from pH and PCO_2 but must be calculated from analysis of the biochemical changes in the cell (Hultman & Sahlin, 1980; Sahlin, 1978).

Transmembrane fluxes of H^+, OH^- and HCO_3^- also affect the hydrogen ion balance of the cell. These transmembrane fluxes depend upon the type (metabolic or respiratory) and the degree of the acid base disturbance (Hultman & Sahlin, 1980; Aicken & Thomas, 1977). However, Sahlin (1978) demonstrated that during short term exercise, the accumulation of lactate and pyruvate in blood is linearly related to the accumulation of the hydrogen ions indicating that the transmembrane fluxes of H^+ and HCO_3^- under these conditions are small.

The buffer capacity of muscle has been sought in vivo and in vitro by manipulation of both volatile (CO_2) and nonvolatile acid. While respiratory effects following manipulation of CO_2 concentrations permit observations on the intact cell, CO_2 is metabolically active and not only influences metabolic reactions but perhaps even cell buffers and acid production (Larsen & Burnell, 1978). Larsen and Burnell (1978) suggest the use of homogenates titrated with either CO_2 or mineral acids. This may be the only way in which mineral acids can be used to measure total potential intracellular buffer capacity because of membrane barriers to the electrochemical equilibrium distribution of hydrogen ion.

There has also been much research with little agreement as to the various intracellular buffers and their corresponding capacities (Hultman & Sahlin, 1980; Larson & Burnell, 1978; Sahlin, 1978). In general, the individual contributors to overall buffering power of the muscle, from largest to smallest, are suggested to be muscle protein; bicarbonate; histidine related compounds such as carnosine and anserine; and, phosphates (Sahlin, 1978).

Bate-Smith (1938) estimated the buffering of intracellular protein to be 15 Sl, while Woodbury (1965) estimates the buffering of protein to be 17-37 Sl. However, there has been little additional research relating buffering capacity and protein so this concept remains speculative (Sahlin & Henriksson, 1984).

Direct measurement in muscle shows that bicarbonate (HCO_3^-) concentration decreases from 10.2 mmol * l^{-1} at rest to about 3 mmol * l^{-1} at the end of exhaustive exercise (Sahlin, 1978). This decrease corresponds to an equivalent uptake of hydrogen ions (Hultman & Sahlin, 1980). Thus, bicarbonate was calculated to contribute approximately 12.0 Slykes to the total buffering capacity of the muscle (Sahlin, 1978).

Davey (1960) postulated that the histidine-containing dipeptide carnosine, found principally within fast twitch fibers could contribute up to 40% of total buffering. Parkhouse (et al., 1983) discovered buffering capacity was significantly related to carnosine ($r = 0.69$), anaerobic performance ($r = 0.60$), and % fast twitch ($r = 0.44$) in anaerobically trained athletes. Parkhouse (et al., 1983) futher suggested that carnosine accounted for 48% of the variance in buffering capacity. On the other hand, Sahlin and Henriksson (1984) argue that even if a maximal buffering power of carnosine is assumed (molar buffer power = .575), the contribution of carnosine is only 2-4 Slykes or 7% of total buffering capacity.

Sahlin (1978) suggests that creatine phosphate (CP) depletion contributes 38% of the total buffering capacity. The breakdown of creatine phosphate causes an uptake of hydrogen ions. Since there is a higher content of CP in high glycolytic or fast twitch muscle, buffering capacity in these fibers may be enhanced (Sahlin & Henriksson, 1984).

Castellini and Somero (1981) observed the highest nonbicarbonate buffering capacities (BC) in muscle capable of high burst glycolytic function (fast twitch) from a variety of terrestrial and marine animals. Strong correlations were found to exist between BC and myoglobin ($r = 0.69$) and, BC and lactate dehydrogenase ($r = 0.54$). However, these correlations were not indicative of a strong causal relationship.

To date only a few studies of the buffering capacity in human subjects have been performed (Sahlin & Henriksson, 1984). There has been more research on the buffering capacities of skeletal muscle of animals (Castellini & Somero, 1981; Larsen & Burnell, 1978; Burton, 1978; Heisler & Piiper, 1972; Davey, 1960). The range of buffering capacity is large, from 15 to 85 Slykes. The discrepancies may lie in the methods used, the muscle fiber type sampled or the degree of training. Furthermore, if buffering capacity is determined in vitro by titration of muscle homogenates, the extracellular compartments become mixed with the intracellular compartment which may change the buffer value (Heisler & Piiper, 1972).

Parkhouse (et al., 1985) examined buffering capacity in deproteinized skeletal muscle of elite athletes and found the highest values to occur in rowers (31.7) and 800 meter runners (30.0) when compared to the untrained group (21.3). Furthermore, the buffering capacity of marathoners (20.4) was not significantly different from the untrained group.

Sahlin and Henriksson (1984) also examined buffering capacity and lactate accumulation in anaerobically trained and untrained men. Calculation of buffering capacity was made from a derived formula based on muscle lactate and pH at rest

and after exercise. The calculation was defended as an accurate predictor of buffering capacity because at isometric contractions exceeding 40% MVC the muscle exists as a closed system and all the produced lactate and H^+ are trapped within the muscle. Utilizing this calculation for buffering capacity, the trained men had a buffering capacity of 193.9 (mmoles * pH^{-1} * kg^{-1} dry weight) which was significantly higher ($p < 0.05$) than the untrained men (164.3). Additional findings of this study indicated that the trained subjects accumulated less muscle lactate and had a greater pH decrease after isometric contraction to fatigue than the untrained subjects. This seems to contradict the idea that a higher buffering capacity in the trained subjects would be important to permit an increased lactic acid generation and anaerobic energy delivery before muscle pH reaches a limiting level. They suggest that other factors such as CrP and ATP depletion may be the cause of fatigue in their experimental situation.

Sprint Training

Mechanisms which permit endurance-trained athletes to perform at higher rates and for longer durations than untrained individuals or sprint trained athletes have been identified. However, the mechanisms that allow sprint-trained athletes to perform at much higher rates are not as clearly defined (Parkhouse & McKenzie, 1984). Parkhouse et al. (1983) have shown sprint-trained athletes have superior performances on anaerobic exercise of high intensity and short duration than do endurance trained or untrained subjects.

The effect of high speed, short intermittent sprint runs on the enzyme activity and muscle strength in man was investigated by Thorstensson et al.

(1975). Results indicated an increase in thigh circumference, MVC and scores on the Sargent jump test. Three of the four subjects also improved in the Margaria sprint test. The muscle enzyme activities of Mg^{2+} stimulated ATPase, myokinase, and creatine phosphokinase increased 30, 20 and 30 percent, respectively. Similar effects were observed by Eriksson et al. (1973) who demonstrated a 30% increase in succinate dehydrogenase and a 83% increase in phosphofructokinase capacities after a 6 week high intensity training program conducted with 11 to 13 year old boys.

Enzyme activities in rat tissue exposed to similar sprint training programs have also shown to increase. Lactate dehydrogenase and fumerase activity increased 15% and 42% respectively (Hickson et al., 1976); hexokinase, citrate synthetase, glycogen phosphorylase, triphosphate dehydrogenase and creatine kinase significantly increased (Staudte et al., 1973); and finally, increases in phosphofructokinase, phosphorylase and pyruvate kinase activity were also observed (Saubert et al., 1973).

The changes in the glycolytic capacity of human muscle appear to be more specific to fast twitch fibers after anaerobic training (Fox & Mathews, 1981; Fink et al., 1975). Costill et al. (1976) examined skeletal muscle enzymes and fiber composition in male and female track athletes. The results show that fast twitch fibers occupy a greater area in sprinters, shot-putters and discus throwers. The majority of research suggests that there is no interconversion of fast and slow twitch fibers as result of training (Fox & Mathews, 1981; Eriksson et al., 1973; Gollnick et al., 1972). However, Jansson et al. (1978) have demonstrated that the percentage of fast twitch fibers increased while the percentage of slow twitch

fibers decreased after 7 to 13 weeks of high intensity anaerobic training. The opposite was found to occur for aerobic training.

A decrease in lactate levels at the same exercise intensity were found to occur after anaerobic training (Boobis et al., 1983). A drop of muscle lactate from 53.2 to 14.3 (mmol * kg^{-1} dry weight) after maximal exercise following a 6 week training period was observed. Karlsson et al. (1972) showed a decrease in muscle lactate concentration from 6.6 to 2.9 (mmol * kg^{-1}) at a 150 W, submaximal work level after 7 months of training. Fox et al. (1977) found a decrease in net lactate levels following 8 weeks of sprint training. Similar results were attained by Saltin et al. (1976) in which blood lactate concentration after four weeks of one legged training were decreased at the same relative workload. Furthermore, Saltin et al. (1976) observed lower muscle lactate levels and lower release of lactate after prolonged submaximal exercise following training for 4 weeks with one-legged sprint or endurance training. However, peak blood lactate concentration was enhanced with sprint training only.

Subsequently, pH levels in the muscle are also altered by sprint training. Costill et al. (1983) discovered a lower intramuscular pH in sprint-trained athletes after maximal exercise when compared to endurance-trained individuals. Muscle pH was found to be higher in anaerobically trained subjects (6.80) than untrained subjects (6.61) after maximal isometric contraction to fatigue (Sahlin & Henriksson, 1984). These results suggest a higher sequestering ability for hydrogen ion in sprint trained athletes.

The capacity of the phosphagen (ATP-PC) system has been shown to increase after anaerobic training as well. Muscle ATP stores were increased approximately

25% (from 3.8 to 4.8 mM * kg^{-1} of wet muscle) following a 7 month training program (Fox & Mathews, 1981; Karlsson et al., 1972). Creatine phosphate was shown to increase by approximately 40% after four months of sprint training (Eriksson et al., 1973).

Thus, the adaptation of skeletal muscle to sprint training may occur to a greater extent in the enzyme activities of glycolysis and may be more pronounced in the fast twitch and fast oxidative glycolytic fibers of the muscle. Furthermore, the muscle produced less lactic acid at the same relative intensity after training resulting in a increased pH during anaerobic work. Peak blood lactate was also increased and a higher pH in muscle after maximal exercise in sprint trained subjects, which may indicate an enhanced ability to sequester H^+ during periods of high lactic acid production. The combined effect is a increase in glycolytic energy production and an enhanced ability to perform at higher intensities.

Summary

The energy requirements during anaerobic exercise are supplied mainly by anaerobic glycolysis. As previously discussed, the result of anaerobic glycolysis is the production of lactic acid and a subsequent release of H^+ and a decrease in pH. The reason for the heightened ability of the sprint-trained athlete to perform anaerobic work may be due to an enhanced buffering capacity within the muscle. Sahlin and Henriksson (1984), and Parkhouse et al. (1985) have shown that sprint-trained athletes have higher buffering capacities than either marathoners of non-athletic controls. However, there has been no research directly linking the effects of sprint exercise on intramuscular buffering capacities.

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Appendix C

EXPERIMENTAL STUDIES ON THE MEASUREMENT OF PH AND BUFFERING CAPACITY IN SKELETAL MUSCLE: A PILOT PROJECT

Introduction

The measurement of the intracellular response to and capacity for increased energy production has provided a challenge to researchers in the past. Recently, refinements to techniques have provided reliable and valid measures of some of these variables. Various methods have been applied in an attempt to understand the acid-base balance within the muscle cell. The measurement of intracellular pH reflects the hydrogen ion activity within the cytoplasm and is important under conditions of acidosis during intense exercise. The ability to resist this increased hydrogen ion activity has been termed buffering capacity and the measurement unit (Slyke) is the amount of acid that is required to change the pH by one unit in a gram of muscle (vanSlyke, 1922).

There are two methods of measuring pH: direct and indirect. Direct methods include the impaling of the muscle cell with an electrode to determine pH. This method has been used successfully on various animal tissue cells but has not yet been applied to human tissue (Ellis & Thomas, 1976; Aicken & Thomas, 1975). This is due to numerous problems such as disruption of the cell membrane which may affect the pH of the cytoplasm and the small cell size of human skeletal muscle in comparison to the size of the measurement electrode.

Several indirect techniques of measuring intracellular pH have been attempted, each with inherent advantages and disadvantages (Roos & Boron, 1981). These include distribution of weak acids or bases (DMO, nicotine), nuclear magnetic resonance and the homogenate technique. The distribution of weak acids or bases has been widely reported in the literature on animal skeletal

muscle, however, there is one account of this technique being used on human skeletal musle (Hermansen & Osnes, 1972). Similarly, there is only one report of pH being measured by nuclear magnetic resonance (Mole et al., 1985). The most widely reported and one of the oldest methods is the homogenate technique (Costill et al., 1982). The major problem associated with this technique is that the pH measured is a composite of the extra and intra-cellular components of the muscle and is more aptly a measure of the intramuscular pH as opposed to intracellular pH.

Buffering capacity (BC) has been investigated in recent literature to determine the tolerance of skeletal muscle to an increased hydrogen ion content. BC has been applied to various muscle tissue assays such as deproteinized muscle (Parkhouse et al., 1985) and nonbicarbonate muscle homogenates (Castellini & Somero, 1981). There are no reports of buffering capacity measures of the total intracelluar buffering process. However, higher buffering capacity of animal and human skeletal muscle has been reported in muscle capable of high glycolytic function (Parkhouse et al., 1985; Castellini & Somero, 1981).

Buffering capacity of skeletal muscle is necessary to tolerate high amounts of lactate generated hydrogen ion under conditions of intense muscular work. Thus, the purpose of the following project was to develop the homogenate technique of determining intramuscular pH and buffering capacity in the animal model. The specific purposes were:

1. to determine resting pH and buffering capacity of mouse skeletal muscle.
2. to determine the time component involved in sacrificing and extraction of the muscle sample.
3. to determine pH at rest and after exercise in rat skeletal muscle.

4. to examine the effect of atmospheric air contamination on pH measurement.
5. to determine the effect of anaesthetic on resting pH and buffering capacity in rat skeletal muscle.
6. to determine the effect of freezing on the weight of muscle samples.
7. to determine the difference between non-bicarbonate and total buffering capacity.

Experiment 1: Determination of Resting pH and Buffering Capacity of Mouse Skeletal Muscle.

The purposes of this experiment were to measure the pH and buffering capacity of mouse skeletal muscle utilizing the homogenate technique, and to determine the amount of time required to extract and freeze the tissue sample after death.

Methods

Two male mice (Charles River Laboratories strain) were sacrificed via concussion and cervical dislocation. The hindlimb muscle was exposed and multiple samples were excised as quickly as possible. The tissue was then weighed, frozen in liquid nitrogen and wrapped in tinfoil for storage. Total time was recorded from death to freezing and also the time from sectioning of the muscle until it was frozen.

The frozen tissue was weighed and then homogenized in a solution of 145 mM KCl + 10 mM NaCl + 5 mM iodoacetic acid (IAA). Further biochemical reactions were eliminated with the addition IAA (Sahin et al., 1981; Sahlin, 1978).

Homogenate solution was added until a 10 ml * g^{-1} dilution was acheived (Costill et al., 1982). Samples were ground in a 5 ml glass tissue grinder until finely minced. pH was determined by insertion of a microelectrode directly into the homogenate in the tissue grinder. Argon gas was continuously bubbled through the homogenate for mixing. pH was read when stabilized (approximately 2 minutes). Buffering capacity was then determined by a modification of the Bate-Smith method (1938) used by Catellini and Somero (1981). pH was initially adjusted to 7.00 with 0.10 N NaOH and repeated titrated (3 times) to 6.00 with 0.01 N HNO_3. pH was allowed to stabilize for about one minute after each addition of acid (Albers & Vaupel, 1981). Buffering capacity in Slykes was defined as the number of umoles of acid per gram gram of tissue required to change the pH by one unit (vanSlyke, 1922). All measures were taken at 25 degrees Celcius since buffering capacity is independent of measurement temperature over a wide range (10 - 37 dgrees Celcius; Castellini & Somero, 1981).

Results

The time from death until the freezing of the first sample from mouse #1 and #2 was 1:36 (min:s) and 1:56, respectively. The time from extraction to freezing was 26s and 52s for mouse #1 and #2, respectively (Table 1). The mean resting pH was 6.83 and the mean buffering capacity was 41.55 Slykes (Table 2).

Table 1. Weight of Muscle Sample and Time From Death and From Biopsy to Freezing.

Sample	Mouse #	Weight of Sample (mg)	Time From Death to Freezing (min:s)	Time From Biopsy to Freezing (min:s)
1	1	10	1:36	0:26
2	1	20	2:16	1:22
3	1	30	3:15	2:15
4	1	60	3:56	2:55
5	1	50	4:33	3:42
6	1	20	5:33	4:33
7*	1	30	8:50	7:50
8*	1	10	9:03	10:04
9	2	70	1:56	0:52
10	2	40	3:26	2:22
11	2	30	4:00	2:57
12	2	80	5:29	4:26

* = forelimb muscle

Table 2. Resting pH, Titration and Buffering Capacity of Mouse Hindlimb and Forelimb Muscle.

Sample	Mouse #	Resting pH	Titration (ml) 1	2	3	Buffering Capacity (Slykes)
1	1	6.97	0.175*	0.174	-	58.30
2	1	6.84	0.249	0.245*	0.244	40.80
3	1	6.98	0.064	0.066*	0.066	33.00
4**	1	6.66	0.152	0.146	0.150	50.00
5	2	6.71	0.245*	0.245	-	35.50
6	2	6.74	0.131*	0.129	0.132	32.80
7	2	6.92	0.354	0.324*	0.324	40.50
Mean		6.83				41.56
SD		0.13				9.49

* = Titration used for calculation of Buffering Capacity.

** = Forelimb muscle.

Discussion

The results show that to freeze muscle samples after death takes considerable time during which further metabolic reactions may occur within the muscle sample. However, the only way to shorten this time would be to anaesthetize the animal and extract the tissue from the live animal.

The mean resting pH for mouse soleus muscle reported by Aicken and Thomas (1977) was 7.07 with a range of 6.97 to 7.22. The present results on mixed hindlimb and forelimb muscle were slightly lower which was probably due to different methodology and time delay for extraction and freezing in the present study. Aicken and Thomas (1977) used direct impaling of the soleus with a recessed tip micro-electrode and measured pH in the excised muscle suspended in normal ringers solution.

Buffering capacity of mouse skeletal muscle was calculated by Aicken and Thomas (1977) based on the Henderson-Hasselbach equation. The results produced a mean non-CO_2 buffering capacity of 45 mequiv H^+ * pH $unit^{-1}$ * l^{-1}. If comparisons can be made using the homogenate technique, the buffering capacity measure of 41.55 in the present study is similar.

Experiment 2: Determination of pH in Rat Skeletal Muscle at Rest and After Swimming.

The purposes of this investigation were: to determine the pH of rat skeletal muscle at rest and after exercise; and to determine whether atmospheric contamination or liberation of CO_2 as a result of grinding in an open vessel would affect the pH.

Methods

Six male Wistar rats (mean weight = 180 g) were subjected to a brief swim in 33-35 degrees Celcius water to determine swimming ability. All rats showed equal swimming ability and then were assigned to either group R to measure resting pH or group S to measure pH in response to a brief swim with a weight (5% of body weight) attached to the base of the tail.

The animals in each group were sacrificed by concussion with subsequent cervical dislocation. The gastrocnemius muscle of one limb was quickly isolated and sectioned into two samples. The paired samples were homogenized in separate tissue grinders containing 10 volumes of a salt solution of 145 mM KCl, 10 mM NaCl and 5 mM IAA for determination of pH as previously described (Costill et al., 1982). One of the homogenates was ground while sealed with parafilm to prevent contamination with ambient air and any liberated CO_2 during grinding. The other homogenate was left exposed. The grinding was carried out at room temperature while all pH measures were conducted at 37 degrees Celcius. Gaseous nitrogen was bubbled continuously through the homogenate for mixing.

Results

The rats were able to complete approximately 10 minutes of swimming before signs of tiring. The mean intramuscular pH at rest and after exercise was 6.72 and 6.91, respectively (Table 3). The effect of sealing the tissue grinder during grinding and measurement of pH resulted an increase of only 0.05 pH units at rest and 0.02 after exercise. This includes only those samples that were immediately ground. The two samples that were left immersed in the homogenate solution for several minutes before grinding exhibited a substantial increase in pH over their paired samples.

Table 3. Rat Intramuscular pH at Rest and After Swimming.

Rat #	Condition	Sample #	Homogenate Procedure	Muscle Sample Weight (mg)	pH Initial	pH Final
1	Rest	A	Exposed	30.0	6.60	6.60
		B	Sealed	40.0	6.62	6.62
2	Rest	A	Exposed	40.0	6.62	6.63
		B	Sealed	40.0	6.77	6.77*
3	Rest	A	Exposed	60.0	6.86	6.83
		B	Sealed	50.0	6.86	6.84
4	Swim	A	Exposed	60.0	6.91	6.90
		B	Sealed	40.0	6.89	6.89
5	Swim	A	Exposed	30.0	6.86	6.84
		B	Sealed	40.0	7.01	6.96*
6	Swim	A	Exposed	60.0	6.88	6.86
		B	Sealed	50.0	6.91	6.88
Mean at Rest		A	Exposed		6.69	
		B	Sealed		6.75 (6.74)**	
Mean after Swim		A	Exposed		6.88	
		B	Sealed		6.93 (6.90)**	

* = These homogenates were allowed to sit in the cocktail without grinding for approximately 5 minutes.

** = This mean for pH does not include those samples described above (*).

Discussion

The mean resting pH was 6.74 which was slightly higher than the mean resting pH of 6.64 reported by Rooth (1966) for rat hindlimb muscle. However, both values are much lower than the mean pH of 6.89 in diaphram muscle reported by Heisler and Piiper (1972) and 7.14 in cardiac muscle of the rat (Ellis & Thomas, 1976). The discrepancies may result from the different methods used to measure pH and to the method of sacrificing or sampling.

The lower pH at rest than after exercise was attributed to to the method of sacrifice. The rest group had severe involuntary contractions of the limbs after concussion whereas, following the swim, the concussed rats exhibited no contractions and seemed to be more relaxed due to the tiring effect of the exercise. An alternative method of sacrificing or use of an anaesthetic or muscle relaxant prior to death seemed warranted.

pH was only slightly altered when exposed to ambient air during grinding. There was only a 0.05 and 0.02 increase in pH with sealing of the grinding chamber which was within electrode measurement error. It was interesting to note that pH climbed considerably in two samples that were allowed to sit in the homogenate cocktail while sealed for several minutes prior to grinding. This occurred in both the swim and rest conditions. This increased pH may be due to further metabolic reactions that may have occurred before grinding suggesting that it is important to grind the samples immediately.

Experiment 3: Determination of pH and Buffering Capacity in Anaesthetized Rat Skeletal Muscle.

The purposes of this investigation were: to determine whether resting muscle pH values close to pH 7.00 could be attained by
use of an anaesthetic prior to muscle extraction; and, to measure buffering capacity of skeletal muscle.

Methods

One Long-Evans hooded rat (Charles River) was anaesthetized with 40 mg * kg^{-1} of nembutal (sodium pentobarbital). Following unconciousness, the gastrocnemious muscle of the rat was extracted and sectioned into 9 samples that were quickly frozen in liquid nitrogen and wrapped in tin foil for storage at -80 degrees Celcius. The rat was later sacrificed by cervical dislocation.

pH was determined via the homogenate technique as previously described (Costill et al., 1982). Buffering capacity was measured as the number of umoles of 0.01 N HCl required to titrate 1 gram of muscle tissue over the pH range of 7.00 to 6.00. NaOH (0.10 N) was used to raise the pH to 7.00 after each titration.

Results

The mean pH and buffering capacity were 7.09 and 50.96 umoles HCl * pH^{-1} * g^{-1}, respectively (Table 4).

Table 4. pH and Buffering Capacity at Rest in Anaesthetized Rat Skeletal Muscle.

Sample #	Weight (g)	pH	Titration (ml)	Buffering Capacity (Slykes)
1	0.091	7.05	0.418	45.82
2	0.050	7.32	0.260	52.00
3	0.061	7.05	0.361	59.18
4	0.059	6.87	0.319	59.15
5	0.056	7.05	0.257	45.89
6	0.055	7.13	0.307	55.82
7	0.055	7.13	0.287	52.18
8	0.059	7.13	0.244	41.36
Mean	0.061	7.09	0.310	51.42
SD	0.010	0.13	0.060	6.58

Discussion

The higher resting pH value discovered in this experiment can be attributed to the use of the anaesthetic which allowed extraction of the muscle prior to death without spasm. Resting pH values shown previously (Experiment #3) in unanaesthetized muscle was 6.72 compared with 7.09 in the present study. The lower pH was previously attributed to involuntary contractions observed after death which were eliminated with use of the anaesthetic.

Inconsistency arises in the literature when attempting to measure resting pH of rat muscle. DMO studies show intracellular pH at rest ranging from 6.64-6.90 (Alder, 1972; Heisler & Piiper 1972; Roos, 1971; Rooth, 1966). The direct measurement shows pH to reach 7.14 in rat ventricle muscle (Ellis and Thomas, 1976). However, in using the homogenate technique, Sahlin et al. (1981) showed a resting muscle pH of 7.08 in rat skeletal muscle which supports the present study.

Buffering capacity of rat skeletal muscle is also difficult to compare to the literature. Eckel et al. (1959) reported a non- bicarbonate buffering capacity of 61 meq * pH^{-1} * kg^{-1} cell H_2O whereas Heisler and Piiper (1972) reported a buffering capacity of 68 meq * pH^{-1} * kg^{-1} cell H2O. There are no reports to date of buffering capacity measured via the homogenate method.

Experiment 4: Comparison of Fresh Versus Frozen Weights of Skeletal Muscle.

The purpose of this investigation was to determine whether there is any significant difference between fresh and frozen weights of skeletal muscle.

Methods

Twelve muscle samples were excised from one chicken leg. Each sample was weighed while unfrozen. Subsequently, each sample was frozen in liquid nitrogen and immediately reweighed. A paired t-test was applied to determine any significant difference between groups.

Results

The two groups were found not to be significantly different, $t(11) = 1.47$, $p > 0.05$. The mean weight of the fresh muscle sample group was 0.055, compared with 0.056 for the frozen group. The mean difference between the two groups was only 0.001 (Table 5).

Table 5. Fresh vs Frozen Weights of Chicken Leg Muscle.

Sample #	Fresh Weight (g)	Frozen Weight (g)	Difference
1	0.046	0.045	0.001
2	0.057	0.057	0.000
3	0.072	0.070	0.002
4	0.071	0.072	0.001
5	0.071	0.071	0.000
6	0.043	0.045	0.002
7	0.040	0.040	0.000
8	0.048	0.050	0.002
9	0.032	0.035	0.002
10	0.069	0.069	0.000
11	0.052	0.053	0.001
12	0.064	0.065	0.001
Mean	0.055	0.056	0.001
SD	0.010	0.010	

Discussion

The results of this experiment indicate that muscle tissue utilized in later analyses may be weighed accurately either fresh or frozen. This may allow for some flexibility in experimental procedures.

Experiment 5: Comparison of Non-bicarbonate and Total Buffering Capacity.

This experiment was conducted to determine if there was any significant difference between non-bicarbonate and total buffering capacity.

Methods

Four Long-Evans hooded rats (mean weight = 409 g) were anaesthetized with somnotol (sodium pentobarbital) and samples were extracted from the gastrocnemius muscle. The samples were quickly frozen in liquid nitrogen and wrapped for storage at -80 degrees Celcius.

The samples were later separated into two pieces and weighed separately. One sample was immediately homogenized in 145 KCl + 10 NaCl + 5 IAA mixture of 10:1 dilution for determination of pH and buffering capacity as described earlier (Costill et al., 1982). However, gaseous nitrogen used for mixing was eliminated in an attempt to achieve stable titrations. Thus, mixing was done manually. The second sample was homogenized using the same method, however, the samples were allowed to sit in the titration vessel for exactly 30 minutes at ambient temperature. This allowed for the release of bicarbonate from the mixture (Albers & Vaupel, 1981; Larson & Burnell, 1978). Subsequently, buffering capacity was calculated as the umoles of HCl * g^{-1} * pH^{-1}.

Results

The non-bicarbonate and total buffering capacity groups were found to differ, $t(3) = 2.85$, $p < 0.05$. The mean buffering capacity for the nonbicarbonate samples was 45.50 as compared to 55.50 (umoles HCl * pH^{-1} * g^{-1}) in the total buffering group (Table 6).

Table 6. Comparison of Non-bicarbonate (NB) and Total (T) Buffering Capacity of Rat Muscle.

Sample #	Type	Weight (g)	Buffering Capacity (Slykes)
1	T	0.038	59.84
	NB	0.043	47.21
2	T	0.023	52.61
	NB	0.028	48.93
3	T	0.018	56.11
	NB	0.030	36.00
4	T	0.042	56.43
	NB	0.018	49.44
Mean	T	0.030	55.50
SD		0.010	2.95
Mean	NB	0.030	45.40
SD		0.010	6.34

Discussion

These results indicate a significantly different buffering capacity can be attained on similar muscle tissue samples using two different methods. Larsen & Burnell (1978) left homogenized sample sit for thirty minutes after grinding to eliminate any bicarbonate ions from the mixture. Bate-Smith (1938) allowed one full hour before titrating the samples. Also, Albers and Vaupel (1981) poisoned the homogenate to pH 5.00 for thirty minutes before titrating. These different methods were followed in an attempt to release bicarbonate and CO_2 from the sample thus producing a non-bicarbonate measure of buffering capacity. Although there are limited reports of total buffering capacity measures in the literature, it seems to follow that if buffering capacity is a measure of the total buffering ability of the muscle, it should include all potential buffers in that system. Thus, buffering capacity measures which include the bicarbonate buffering system may be a more valid measure of the total buffering potential of the muscle.

Observations from the present and previous experiments showed that the titrations of the homogenates measured immediately after grinding were unstable and that each subsequent titration was less than the preceding one. This would indicate a loss of buffering power over repeated titrations. This was thought to be due to the use of gaseous nitrogen for mixing which may have had an negative influence on the buffering process. However, this instability to reproduce the same titration values was observed in the present study in which no nitrogen was used. However, the titrations for the non-bicarbonate homogenates were very stable. Thus, it may be the emission of the bicarbonate/CO_2 system from the

ground homogenates that caused the loss of buffering power, suggesting that the non-bicarbonate measure may be more reliable. Due to the speed of the bicarbonate/CO_2 reaction, it may be erroneous to assume that total buffering capacity can be acheived with homogenate technique due to the time considerations in grinding and titrating the samples.

Summary

1. Intramuscular pH and buffering capacity were determined in mouse skeletal muscle utilizing the homogenate technique. A significant time delay occurred between sacrificing of the animals and freezing of the samples which may affect pH.
2. Muscle pH was determined in rat gastrocnemius muscle at rest and after a brief swim. pH was shown to be lower in the resting state which was attributed to the method of sacrifice. Buffering capacity was found to be similar in the resting and exercised state. Also, grinding of the tissue in a closed versus an open vessel had no effect on subsequent measures. Furthermore, it was discovered that tissue should be ground immediately after immersion in the homogenate solution for an accurate measure.
3. The use of an anaesthetic (sodium pentobarbital) prior to extraction of the muscle sample produced a more valid resting pH measure and had no effect on buffering capacity.
4. Weighing of muscle samples in either the fresh or frozen state showed no significant differences.
5. Total buffering capacity was found to be significantly higher than non-bicarbonate buffering capacity which was suggested to be due to the bicarbonate buffering process.

6. However, due to unstable titrations and time differences in preparation of the homogenate, total buffering capacity may not be a reliable or valid measure.

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