

## FORMAT FOR RESEARCH PAPER

### Open a new page on Microsoft Word

- Page setup: File tab → Page setup → Adjust margin for top, bottom, left and right to 1" → Click "OK"

### Title

- Font: Times New Roman (apply to whole document)
- Font size: 12
- Bold & center title on page

### Name

- Enter: first & last name
- Enter in italicize: *Department of Biological Science*
- Enter: Saddleback College
- Enter: Mission Viejo, CA 92692
- Center name on page

### Abstract

- Cut & paste abstract on to page
- Font size: 12. Make sure that Abstract is in bold and justify on page.
- Single spaced (apply to whole document)

### Introduction, Materials & Methods, Results, and Discussion

- Leave a space after your Abstract and then insert columns.
- Inserting columns: Format tab → Columns → Click on the "two columns" box → Adjust the width to 3.1" and the spacing to 0.3" → Apply it to "this point forward" → Click "OK"
- Cut & paste Introduction, Materials & Methods, Results, and Discussion on to page.
- Font size: 12 (for title) and 10 (for text). Make sure that it this single spaced and justify.
- If you need to insert a table, place it at the end of the Results section (make sure you place the cursor at the place where you want to input your table).
- Inserting table: Table tab → Insert → Table → Enter in the number columns and rows needed → Click "OK"
- Make sure to label your table by placing the table and number in bold and the text in italicize. (Ex: **Table #.** *text...text...text*).
- If you need to insert a graph, place it at the end of the Result section and after your table.
- Cut & paste your graph from Microsoft Excel only.
- If you need help generating a Microsoft Excel graph then refer to Statistical Analysis & Graphing Worksheet.
- Make sure to label your graph by placing the figure and number in bold and the text in italicize. (Ex: **Figure #.** *text...text...text*).
- If you need to insert a picture, place it at the end of the section from which you reference it.
- Make sure to label your picture by placing the figure and number in bold and the text in italicize. (Ex: **Figure #.** *text...text...text*).
- Numbering for figure(s) and graph(s) are the same. Graphs and pictures are numbered as figures. Always start with figure #1

### Literature Cited

- Place each citation in alphabetical order according to the first author's last name.
- Italicize journal and book titles.
- If you need help with the citation then refer to *The Student Handbook for Writing in Biology*.

## SAMPLE RESEARCH PAPER

### **Comparisons of Lactate Accumulation after Extensive Exercise and Premature Lactate Accumulation after Lactate Injection in *Hemidactylus frenatus***

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**Anaerobic glycolysis is a major source of energy for activity in reptiles. In this experiment, lactate and premature lactate accumulation in house gecko (*Hemidactylus frenatus*) was measured by the time it took the geckos to reach exhaustion, righting response and change in respiration rates. Lactate accumulation was measured after extensive exercise on the treadmill and injection of 0.9% NaCl (saline) solution, while premature lactate accumulation was measured after injection 80 mM solution of lactic acid which was adjusted to 300 mOSM of NaCl (lactate) solution. The result showed that the null hypothesis was rejected and significant differences were found among the three groups. Further test determined that significant differences were found during the time it took for the geckos to reach exhaustion. The differences were among the lactate injection and no injection group, and the lactate and saline injection group.**

#### **Introduction**

Glycolytic metabolism is utilized by many ectothermic vertebrates as a way of producing energy which is maintained during daily activities. It is supported by substantially elevated aerobic and anaerobic metabolic activities which work concurrently with one another. Since most ectotherms are limited in their ability to utilize aerobic metabolism additional support is provided by an anaerobic metabolism to rapidly produce ATP in the muscles. While utilization of anaerobic metabolism can provide the opportunity for higher levels of performance, it can also lead a variety of metabolic consequences. Exhaustion, rapid fatigue and loss of activity are metabolic consequences caused by glycogen depletion and lactate accumulation. Muscle glycogen depletion can limit the intensity and duration while lactate accumulation can cause incapacitated results. Therefore a recovery period following intense activity is needed to allow adequate break down of lactic acid build up within the muscles.

Laboratory research on lactate production and removal during activity and recovery are significantly different among mammals and reptiles (Gleeson, 1996). In mammals, lactate is quickly transported and oxidized from glycolytic muscle fibers to oxidative muscle fibers (Brooks, 1986). In contrast, reptiles tend to rely upon glycolytic pathways which results in greater amounts of lactate production over short-term intervals. Numerous studies have indicated that reptile

can maintain high anaerobic scope, maximal rate of lactate formation, and anaerobic capacity, total lactate formed during exhaustive exercise or activity. Although some lactate can accumulate in less intense activities most lactate accumulation is produced during intense exercise such as escaping from predators, foraging for food and agonistic encounters with other ectotherms (Pough and Andrews, 1985). Previous experiments have indicated that natural activity in the field by *Anolis bonairensis* (Bennett et al., 1980) and *Dipsosaurus dorsalis* (Bennett and Dawson, 1972) involved a degree of anaerobic metabolism and resulted in the accumulation of lactic acid. But little research has shown whether premature lactate accumulation, caused by lactate injection, will produce similar results. The main aim of this experiment is to study the lactate accumulation and righting response after extensive exercise and lactate injection in average house geckos (*Hemidactylus frenatus*). It is expected that there will be a significant difference between the lactate accumulation and prelactate accumulation.

#### **Materials and Methods**

House gecko (*Hemidactylus frenatus*) weighting 1.49-3.00g (N=9, mass  $2.16 \pm 0.57$ , mean  $\pm$  S.E.M) were purchased from LLL Reptile Company in Oceanside, California. The geckos were housed in a glass aquarium with bark substrates. The environment was kept between 75-80°F by having a

24-h heat lamp outside of the aquarium and a heat rock inside the aquarium. They were fed 15 crickets three times week and water was provided accordingly. In order to prevent regurgitation of prior meal during rigorous anaerobic exercises, food was withheld for a 24-h period prior to experimentation and only water was provided.

One week prior to experimentation, each gecko was individually trained to run on a motor-driven treadmill. At the beginning of the session, each gecko ran while incased in a small Plexiglas box. As fatigue set in, the box was removed and the geckos were stimulated to sprint by gentle prodding of their hind limbs and tail. Overall treadmill speed was continuously adjusted to match each gecko's sprinting speed. The environment was kept between 75-80°F by a space heater.

During the experimentation session, each gecko was removed from the aquarium and placed in a separate isolated cage for five minutes prior to treadmill run. This was to prevent miss measurement of respiration rate by allowing each the geckos to reach resting respiration rate. After five minute, resting respiration rate was determined by counting the number of time the thorax raised within a one minute interval. Then each gecko was subjected to a sprint session on the treadmill. A stop watch was used to determine the amount of time that it took to reach exhaustion.

An 80 mM solution of lactic acid that was adjusted to 300 mOSM of NaCl (lactate) solution, and a 0.9% NaCl (saline) solution were mixed in the laboratory prior to injection. Each injection was administered consecutively one day after the first injection or treadmill run (no injection) to allow the adequate breakdown and expulsion of prior solution. Intraperitoneal injection (I.P.) was administered by a 30 gauge (BD Micro-Fine™ IV) insulin syringe. Specific amount of solution was determined according to each gecko's weight by using equations found in the article: *The roles of acidosis and lactate in the behavioral hypothermia of exhausted lizards* by Erica Wagner et al, 1998.

During the experimentation session, each gecko was removed from the aquarium and isolated in order to measure respiration rate. Then each gecko was injected with an appropriate amount of solution. After lactate and saline injections each gecko was subjected to a sprint session on the treadmill. Lactate injection was made prior to saline injection.

As geckos start to show signs of fatigue from the exercise, test for righting response was done by flipping the geckos on their back and observing whether they are able to right themselves on the surface of the treadmill. If they were able to right themselves, the geckos were subjected to further exercise on the

treadmill until they were completely exhausted. When the geckos are unable to right themselves on the surface of the treadmill, they were placed on their back into a clear Plexiglas cylinder. While in the cylinder, the time for righting was measure as the cylinder is slowly rotated. After the geckos were able to right themselves, their recovering respiration rate was determined. Righting response was tested following sprinting session with no injection, with lactate injection, and with saline injection. The mass of each gecko was obtained after the sprinting session where no injection was made.

Results among the three exercise injection groups were compare with repeated-measures one-way analysis of variance (ANOVA) followed by a paired *post-hoc* test. Differences were considered significant at  $P < 0.05$ . All data were expressed as a mean  $\pm$  S.E.M.

## Results

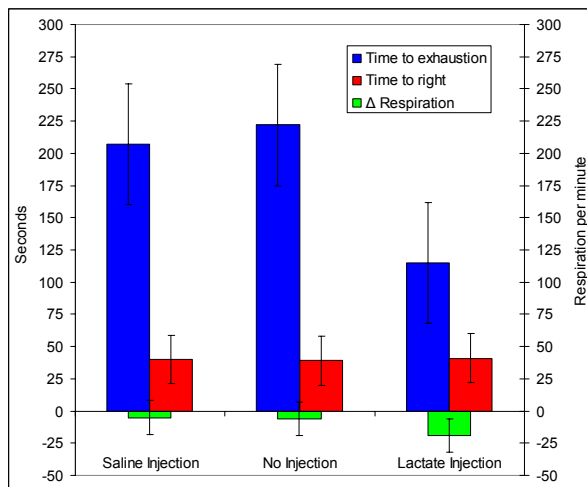
Exhaustion in house geckos is caused by lactate accumulation in muscle and blood. It is brought on by maximal activity endured within a specific amount of time. The time it took to reach exhaustion was determined among house geckos that underwent saline injection, lactate injection and no injection (Table 1). No significant difference (repeated-measure ANOVA) was established between the saline injection and no injection groups. But the time to exhaustion was significantly difference (repeated-measure ANOVA) when lactate injection was compare with saline injection and no injection groups (Figure 1).

Following exhaustion, house geckos appeared thoroughly fatigued and unresponsive for a specific amount of time. Righting response was determined by the amount of time it took for the house geckos to break down the lactate. The time it took for the geckos to right themselves was determined among house geckos that underwent saline injection, lactate injection and no injections (Table 1). No significant difference (repeated-measure ANOVA) was established in righting response among house geckos that underwent saline injection, lactate injection and no injection (Figure 1).

Resting respiration rate prior to experimentation was determined and compared with recovering respiration rate subsequent to experimentation. Resting and recovering respiration rates were determined among house geckos that underwent saline injection, lactate injection and no injection (Table 1). No significant difference (repeated-measure ANOVA) was established in the change in respiration rate among house geckos that underwent saline injection, lactate injection and no injection (Figure 1).

	Time to exhaustion	Time to right	$\Delta$ Respiration
Saline injection	207 $\pm$ 26*	40 $\pm$ 9	-5 $\pm$ 4
No Injection	222 $\pm$ 26**	39 $\pm$ 7	-6 $\pm$ 6
Lactate Injection	115 $\pm$ 12	41 $\pm$ 10	-19 $\pm$ 8

**Table 1.** Table displaying the means $\pm$ S.E.M values for exhaustion time, righting time, and the change in respiration within the three injection groups. \*Statistical significant between saline injection group and lactate injection group. \*\*Statistical significant between non injected group and lactate injection group.



**Figure 1.** Bar graph displaying the means $\pm$ S.E.M values for exhaustion time, righting time, and the change in respiration within the three injection groups. \*Statistical significant between saline injection group and lactate injection group. \*\*Statistical significant between non injected group and lactate injection group.

## Discussion

In the time the time it take to reach exhaustion results showed a significant difference was established when the lactate injection group was compare with saline injection and no injection groups. But the result of the righting time showed no significant different among the three groups. Also no significant difference was established in the change in respiration rate among house geckos that underwent saline injection, lactate injection and no injection. However, resting respiration rate prior to experimentation was less than the recovering respiration rate subsequent to experimentation, this lead to a negative drop in respiration rates.

This experiment indicates that rigorous activity by house geckos many involve a degree of anaerobic metabolism and result in the accumulation of lactic acid. The similarity in lactate accumulation between the lactate injection group and the no injection group suggest that anaerobic metabolism makes a significant contribution to daily activity in this lizard. The *Anolis bonairensis* (Bennett et al., 1980) and *Dipsosaurus dorsalis* (Bennett and Dawson, 1972) experiments support the association of lactic acid formation with vigorous activity under field conditions. Although these anoles may undergo significant bouts of anaerobically supported activity, it is not possible on the basis of these observations to quantify their frequency or energetic contribution. The rate of lactic acid turnover and elimination under these circumstances is not known. The lactic acid may have been formed in only a single burst of activity or may be the result of low-level continuous production. Anaerobic metabolism certainly accounts for only a minor proportion of the total daily energy utilization of these lizards. Anaerobic energy utilization, however, permits a behavioral capacity beyond that provided by aerobiosis. Consequently, its importance cannot truly be measured in caloric or temporal terms alone.

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