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Effects of Alcohol and Training on Exercise Performance and Muscle Recovery Biochemistry in the Brown Anole (Anolis sagrei)

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Introduction

Locomotion in animals plays an essential role in many aspects of survival and successful fitness. An animal depends largely on locomotion to escape predation, feed successfully, avoid or endure aggressive encounters, attract mates, reproduce, and to simply move from place to place. Different species have evolved a variety of morphological and biochemical adaptations to facilitate specific forms of locomotion that enable individuals to move in ways that should maximize energy efficiency. Scientists who study exercise physiology and behavioral energetics have made interesting discoveries that further an understanding of these adaptations.

Reptiles are a particularly well-studied and useful model in exercise physiology because they are easy to study, and exhibit remarkable diversity of morphology and modes of locomotion. Reptiles vary widely in size and shape among different taxa, and their habitats range from primarily arboreal, to marine environments, to terrestrial. They are diurnal or nocturnal, they exhibit different temperature preferences, and they vary in predation strategies. The wide spectrum of behavior even within the squamate lizards, in particular, lends itself well to comparative research into physiological adaptations in locomotion. Limb reductions in some lizards and the sticky toe-pads of other lizards are just a few examples of adaptations for which performance consequences have been investigated (Bennett 1994, Jones 1994).

One particularly intriguing property of lizards is their variation in predation strategies. The majority of lizards fall into two categories: "sit-and-wait" predators and "wide foragers" (Anderson and Karahov 1981). As their names suggest, the first strategy involves short bursts of intense activity requiring anaerobic support for the muscles

involved in sprinting or jumping, whereas the latter involves sustained, moderate, aerobic activity levels. In the lab, endurance capacities of reptiles parallel action in the field for certain species (Garland 1999).   In general, most lizard species tested in exercise physiology studies fall under the anaerobic, "sit and wait" category. In addition to episodic predation events, territory defense and nest building are also energetically expensive activities, although in the field the latter behaviors rarely lead to complete exhaustion (Anderson and Karahov 1981, Hertz et al. 1988, Jones 1994).

Locomotor activity and the requisite muscle contractions are fueled by adenosine triphosphate (ATP) produced by combinations of aerobic respiration, glycolysis followed by lactate production (anaerobic fermentation), hydrolysis of phosphocreatine (PCr), fatty acid metabolism and other less significant pathways (Hochachka and Somero 1984). The relative contributions of energy sources, including carbohydrate, fat, and PCr, vary by animal, activity intensity, and activity duration (Hochachka and Somero 1984, Gleeson 1991, Bennett 1994, Jones 1994). In mammals for example, shorter periods of medium to high activity intensity are sustained primarily with aerobic respiration before anaerobic glycolysis is used. Indeed, aerobic respiration provided as much as 92% of the ATP consumed during 2-3 minutes of moderate to intense activity in humans (Ruben and Battalia 1979). PCr hydrolysis and glycolysis are used in extremely short and intense exercise in all species (Hochachka and Somero 1984, Bennett 1994). In both cases, a decrease in pH (due to lactate presence) and inhibition of actin and myosin by inorganic phosphate (P_i) cause muscle fatigue, which eventually forces animals to stop activity and recover (Bennett 1994, Nelson and Cox 2005). Lizards, as opposed to mammals, rely

more heavily on anaerobic glycolysis; in several studies, 60-90% of the ATP used during 2-3 minutes of moderate to intense exercise was formed anaerobically (Bennett 1994).

There are several possible reasons for this reliance on anaerobic respiration, and its relationship with the common sit-and-wait strategy. First, ectothermic and poikilothermic reptiles do not regulate their body temperature to the extent that endotherms do; without this energetic requirement, their basal metabolic rate, and consequent oxygen intake, is much lower (Hochachka and Somero 1984, Jones 1994). This low oxygen intake logically limits aerobic capacity (Jones 1994). To accommodate low metabolic rates, circulation rates are also low in reptiles compared to mammals, making cardiac output a limiting factor in oxygen consumption (Bennet 1994). Lastly, reptiles are limited by their muscle composition. The three major skeletal muscle fiber types are slow-twitch (Type I), fast-twitch (Type II), and oxidative fast-twitch (Type IIa). Most animals have a combination of all three types, or similar derivatives. Type I muscle fibers contain many mitochondria and are well equipped for aerobic respiration. Lizards specifically have mostly Type II and Type IIa fibers in the leg muscles they use for running or walking (Gleeson and Harrison 1986, Bennett 1994). The lack of mitochondria-rich muscle tissue limits aerobic capacity, and muscles are more likely to rely on an anaerobic biochemical process state during exercise. Given that anaerobic pathways cannot be sustained for long periods of time, these muscle adaptations complement the sit-and-wait predator strategy.

Energy to fuel muscle contraction is stored in the form of glycogen in all types of skeletal muscle and in the liver. The mechanism of aerobic respiration begins with glycolysis, which occurs with the catabolism of glucose into two pyruvates (Nelson and

Cox 2005). In aerobic conditions, pyruvate is then oxidized and continues into the Kreb's cycle, eventually being completely oxidized into CO_2 and H_2O. However, if the muscle cells are operating in hypoxic conditions, such as in reptile muscle during short bursts of intense activity, pyruvate becomes reduced, forming lactic acid (Nelson and Cox 2005). Pyruvate oxidation is a much more energy-efficient process than lactate formation. Complete oxidation of pyruvate produces 30-32 ATP, whereas anaerobic glycolysis produces 2 ATP (Hochachka and Somero 1984, Nelson and Cox 2005, Hancock and Gleeson 2008). Muscle contraction still requires the same amount of ATP; therefore muscles that rely on an anaerobic pathway will deplete glycogen stores much more rapidly than those using an aerobic pathway for ATP production.

Given that most lizard species rely heavily upon anaerobic pathways to fuel locomotion, it is not surprising that many species exhibit physiological mechanisms to more efficiently use excess lactic acid to replenish glycogen stores depleted in skeletal muscle during and after exercise.   In mammals and amphibians, excess lactate is metabolized primarily through oxidation into CO_2 and expired or released as carbonic acid into the blood (Gleeson 1991, Bangsbo et al., 1997, Nelson and Cox 2005). It can also be turned into glycogen hepatically through the Cori Cycle, where lactate is released into the blood stream, transported to the liver and used to make glucose in a process called gluconeogenesis. The glucose can then either be polymerized into glycogen in the liver, or it can be sent back to the muscles to rebuild glycogen stores (Fournier et al. 2002). After long periods of intense exercise, many mammals, including humans and rats, are able to resynthesize glycogen in the actual muscles using lactate, particularly when intense exercise is followed by starvation (Nikolovski et al. 1996). However, this

second location is used rarely in mammals, and in humans total lactate glycogen resynthesis may only account for up to 10% of resynthesized glycogen (Bangsbo et al. 1997).

In lizards, however, this secondary process is the dominant form of lactate removal. In fact, liver glycogen stores play a small role, if any, during recovery in lizard, and glucose production from lactate occurs almost exclusively in the skeletal muscle (Gleeson and Dalessio 1989, Gleeson 1991). Anaerobic glycolysis operates as a closed system within the muscle cells, unlike aerobic respiration where glucose and other metabolites are transported to the muscle from the liver and adipose tissue (Hochachka and Somero 1984). The lack of reliance on liver glycogen stores further supports that lizard skeletal muscle relies more on glycolytic metabolism.

The pathway of gluconeogenesis is effectively the reversal of glycolysis. Some of the steps of glycolysis are highly exergonic, and thereby irreversible, so different pathways have evolved in different tissues to circumvent these steps. In general, the pathway takes 3,4 carbon molecules and generates glucose, which can then be polymerized into glycogen (Gleeson 1991, Bennett 1994, Nelson and Cox 2005). Compared to the oxidation of lactate, this process has very high energetic costs. The pathway requires hydrolysis of 6 ATP, whereas only 2 ATP are generated by anaerobic glycolysis (Hancock and Gleeson 2008). Most animals have a combination of muscle fiber types including Type I oxidative fibers and Type II glycolytic fibers. The aerobic oxidative fibers oxidize lactate during recovery to fuel the glyconeogenesis process, which in lizards also occurs primarily in the Type I and Type IIa fibers (Gleeson 1991). In mammals this glycogen resynthesis occurs in Type II fibers (Bennett 1994), however

results are inconsistent and more research is necessary to identify the details of this process. As a consequence of this aerobic expense, most animals exhibit high levels of oxygen consumption after exercise. The sustained cost after exercise is called the excess postexercise oxygen consumption, or EPOC.

In reptiles, and especially lizards, EPOC is the major energetic cost of exercise. Oxygen consumed following exercise increases with activity duration and intensity, and is more costly than the exercise itself. Animals are fully recovered when most of the accumulated lactate has been removed, and glycogen stores have returned to resting levels. As lizards use lactate as a gluconeogenic substrate rather than oxidizing it, recovery periods in lizards are much longer than in mammals. In fact, it takes several hours for a lizard to recover from exhaustive exercise, when it would take a mammal less than ten minutes (Gleeson 1991, Hancock and Gleeson 2002). To maximize efficiency and limit this costly recovery period, lizards use short intermittent bursts of activity and recover between each burst rather than completely depleting glycogen stores in one longer period (Gleeson and Hancock 2002).

Despite the high costs, glyconeogenesis remains as the primary recovery pathway. It seems to be more important for lizards to replenish the molecule that they rely upon for those short-bursts of high energy than to oxidize the lactate immediately. Behavior and activity in the field are limited by available glycogen and as a result it is advantageous to maintain high levels of glycogen in leg skeletal muscles (Gleeson and Dalessio 1989, Gleeson 1991). Lizards also forage less frequently than mammals, and therefore will not always replace energy stores immediately through feeding as mammals do (Gaesser and Brooks 1980).

Many researchers have worked to further the understanding of the properties of the EPOC and the recovery process. One explanation for the length of recovery period is the dependency on oxygen. After running to exhaustion, preferred body temperature in *Anolis sagrei* decreased during recovery, presumably to prevent hypoxic conditions (Peterson et al. 2003). A lower body temperature slows metabolic rate, making recovery possible with low available oxygen. When exhausted anoles were placed in hyperoxic conditions to recover, preferred body temperature did not decrease below the average (Peterson et al. 2003).

EPOC in the desert iguana (*Dipsosaurus dorsalis*) increases with longer periods and greater intensity of exercise, presumably when anaerobic respiration would be employed (Hancock and Gleeson 2002). Researchers have identified the proportions of fuel sources providing energy for 2-3 min of exercise in the desert iguana as 65% anaerobic glycolysis, 29% phosphocreatine (PCr) hydrolysis, and 2.5% aerobic respiration. When the EPOC was also broken down, they found that 32-50% of the excess oxygen went to PCr repletion, and 30-47% went to glyconeogenesis (Hancock and Gleeson 2008).

In addition to variation in exercise type, activity before exercise also influences post-exercise recovery in the desert iguana. EPOC following exhaustive exercise was diminished in iguanas subjected to brief (15s) periods of activity 30 minutes before exercise (Scholnick and Gleeson 2000). As described above, lizards typically employ intermittent short bursts of activity in the field (Gleeson and Hancock 2002). It appears that intermittent motion not only serves to minimize total glycogen depletion, but also reduces cost of overall activity. Mechanisms of facilitated recovery in sequential activity

could be due to activation of hormones, enzyme up-regulation, or a number of other pathways.

In many animals, endurance or sprint training can improve mechanisms of recovery, lowering recovery rates and improving overall fitness (Holloszy and Booth 1976). Mammals such as rats and humans exhibit an increase in oxidative capacity in red muscle fibers in response to aerobic and anaerobic training. Capacity is increased primarily because of increased enzyme activity, increased number of mitochondria, and enhanced levels of energy substrate. Oxidative enzyme activity levels are the largest contributing factor (Hochachka and Somero 1984). Citrate synthase is one such enzyme, and it catalyzes a condensation reaction in the first step in the Kreb's cycle. It is rate-limiting in ATP production via the oxidation of pyruvate (Nelson and Cox 2005). Consequently, its activity level reflects the oxidative capacity of muscle and is often used to measure response to endurance training. Anaerobic training results in hypertrophy of white fast-twitch muscle fibers, increased glycogen levels, increased enzyme activity per unit muscle mass, and improved buffering capacity. Enzymes affected by training include glycolytic enzymes and phosphocreatine kinases. Lactate dehydrogenase (LDH) is an important enzyme in lactate removal and activity levels increase in response to sprint training (Hochachka and Somero 1984). Enhanced removal of lactate facilitated by LDH, coupled with the improved buffering capacity in trained muscle delays the fatigue caused by lactate accumulation. Muscles can then accumulate more lactate, and maintain aerobic respiration at lower oxygen levels without fatigue (Hochachka and Somero 1984, Holloszy and Booth 1976).

The effects of training have also been studied in many species of fish. Like lizards, most fish skeletal muscle is highly glycolytic, composed primarily of Type II white muscle fibers. Sprint training increases buffering capacity (and protection from lactate) and shortens recovery time (Pearson et al. 1990). When rainbow trout, *Salmo gairdneri*, were trained with 30s of chasing on alternate days for 9 weeks, their energy stores were higher after exercise and during recovery than untrained trout; one mechanism to explain this difference may be that the skeletal muscle in trained fish used more exogenous glucose to replace glycogen stores (Pearson et al. 1990). Endurance training in another fish species (*Leuciscus cephalus*) also resulted in shorter recovery time (Lackner et al. 1988). Trained fish were forced to swim against a current for several days. When exercised to exhaustion, muscle glycogen stores returned to pre-exercise levels more quickly in trained than untrained groups. Rather than an enzymatic change, improvement was likely due to hypertrophy of existing oxidative muscle fibers (Lackner et al. 1988).

In most studies, lizards have shown negative responses in performance and recovery physiology to training. Long-term endurance trained groups of the lizard *Amphibolurus nuchalis* exhibited decreased sprint speed over time, though enzyme activity levels in skeletal muscle increased at the end of an eight week study period where they walked for 30min/day five days/week. The decrease in sprint speed after more than four weeks of training was likely due to observed pathological consequences of training including deteriorated muscle and/or inflamed muscle, and brittle joints. Liver mass increased as time in captivity increased for both trained and untrained individuals indicating possible pathologies (Garland et. al 1987). Similarly, short burst training did

not improve metabolic capacity in the lizard *Sceloporus occidentalis*. Enzyme activity levels and performance, as well as other metabolic characters, did not differ between trained and untrained individuals (Gleeson 1979). However, skeletal muscles in lizards exhibit increased endurance in response to chronic electrical stimulations similar to improvements shown in endurance-trained mammalian skeletal muscle (Gleeson 1991). This suggests that these muscles at least have the potential to respond to training.

Environmental influences beyond training may also affect muscle structure and function. In humans, alcohol has many deleterious effects on skeletal muscle, which can counteract the positive effects of training. In extreme cases, alcoholism can lead to chronic alcoholic skeletal myopathy, which is characterized by selective degradation of Type II muscle fibers (Preedy and Peters 1990). Preedy and Peters (1988) report that chronic alcohol exposure in rats in the form of a liquid diet containing alcohol to represent 36% of the total calories results in muscle mass, protein, RNA, and DNA degradation predominantly in Type II, glycolytic muscle fibers.

Alcohol has immediate and acute effects on skeletal muscle in addition to these long-term changes. At the system level in humans, alcohol affects the level of free glucose in the blood stream due to decreased gluconeogenesis and glycolysis in the liver (Heikkonen et al 1998). When liver glycogen levels are low because of previous activity or starvation, alcohol causes hypoglycemia via inhibition of liver gluconeogenesis by alcohol. When glycogen is plentiful, increased glycogenolysis results in high blood glucose levels (Heikkonen et al 1998). During exercise in humans, muscle and liver glycogen is depleted rapidly. After exercise the little remaining blood glucose is used to restore glycogen levels in the manner discussed above. Heikkonen et al (1998)

established that even a day after alcohol exposure, blood glucose levels post-exercise were significantly lower than in control groups. This shows that the alcohol has lasting effects on carbohydrate recovery metabolism in at least one animal even after the alcohol itself is removed from the system.

Even more intriguing is that alcohol intake before exercise in rats, and after exercise in humans, impairs skeletal muscle glycogen resynthesis (Burke et al. 2003, Peters et al. 1996, Spolarics et al. 1994). In alcohol-exposed rats, lactate levels are elevated during recovery in Type I muscle, but not Type II (Peters 1996). Decreases in lactate removal in red muscle, coupled with high blood glucose levels following intense short exercise indicate inhibition of insulin-mediated glucose uptake by those cells (Spolarics et al. 1994). In humans glycogen levels declined most significantly when alcohol replaced carbohydrate intake in the diet, demonstrating that it cannot be used as a substrate for glycogen resynthesis (Burke et al. 2003).

Few studies have been conducted on the effects of alcohol on the behavior or physiology of lizards, and there have been no studies on its effect on running performance or recovery. Lizards are an ideal model to study training and alcohol effects on an organism adapted for high levels of anaerobic activity. The effect of alcohol on behavior and physiology appears to be rapid; in as few as nineteen days of exposure in the form of a 19% EtOH solution sprayed into their shelters twice daily, individual *Anolis carolinensis* exhibited greater stress response and aggressive lateralization (Deckel 1997). In the present study, I tested the hypotheses that a short training period would influence recovery and muscle biochemistry in the Brown Anole, *Anolis sagrei,* and that two weeks of chronic alcohol ingestion would affect any influence of training on the metabolic

recovery process. I measured the effects using biochemical assays for glycogen concentrations in leg skeletal muscle and liver. I measured activity levels of citrate synthase in leg skeletal muscles to detect changes in aerobic capacity of the muscle as a consequence of training or alcohol exposure.

Anolis sagrei is an iguanid lizard native to Cuba. They are invasive in at least Florida, the Bahamas, Jamaica, and Belize (Schwartz and Henderson 1991). Both males and females are highly territorial. They exhibit behaviors such as head-bobbing, tongue display, tail wags, push-ups and dewlap display to defend territories and court members of the opposite sex. When encountering prey, brown anoles generally retreat quickly when successful to avoid competitors (Schwartz and Henderson 1991). They are also sit-and-wait predators and often consume large prey in relation to body size, therefore considerable time and energy is spent catching, chewing, and digesting food (Schwartz and Henderson 1991). These factors together make *A. sagrei* a good model for studying recovery from intense exercise. *Anolis sagrei* and its congener *Anolis carolinensis* are also both well-studied models for exercise physiology.

Given my hypothesis, I expected that a shorter training regime would not result in the pathological effects demonstrated in longer training periods of 4-6 weeks (Garland et al. 1987, Gleeson 1979) because the time period does not allow for dramatic remodeling of musculature. If deleterious effects did not occur, I expected that trained anoles would have higher activity levels of citrate synthase, and higher glycogen levels in both leg muscles and liver at the end of the two weeks at rest, immediately after exercise, and during recovery. Finally, I expected that alcohol would negatively affect the recovery process as demonstrated previously in rats and humans (Burke et al. 2003, Peters et al.

1996, Spolarics et al. 1994). Although studies show alcohol primarily influences oxidative tissues, glycogen resynthesis occurs in these tissues, and therefore this treatment could affect recovery.

Methods

Study species

One hundred wild-caught brown anoles (*Anolis sagrei*) were acquired from Glades Herps in Florida and housed individually in 12 qt Sterilite® plastic tubs lined with astroturf. I bored holes into the sides to improve airflow and each tub contained a stick for perching, an overturned cup to serve as a retreat, and a water bowl to keep humidity levels high. I fed the lizards two to four crickets twice weekly. On occasions in the winter when crickets were unavailable, I gave them mealworms. I also sprayed each tub at least every other day with distilled water and provided full-spectrum lights with a light-dark cycle of 13.5-10.5h and a daily temperature range of 21-28°C. Before treatment, each group acclimated for at least eight days. In total, the anoles were in captivity for as little as two weeks and at the longest eight weeks before dissection.

Experimental Design

I divided this experiment into two parts. The first part was a three-way factorial comparing the effects of training, recovery time, and alcohol treatment on final running performance and mean muscle and liver glycogen levels. I assessed the effects of each of the independent variables on the dependent variables using separate three-way

randomized-groups ANOVAs with a sample size of six in each treatment. When outputs were nonparametric, I analyzed the data using robust regressions, which accounted for unequal variances. I calculated all statistics using STATA$^{©}$ and used transformations for glycogen concentration data sets which accounted for normalization and homogeneity of variances.

I initially identified four groups of anoles with the different combinations of alcohol and training treatments and randomly assigned anoles to each group. Some anoles were trained for two weeks while others remained sedentary (see training description below). On the final night of training, I exercised each to exhaustion and randomly assigned half as recovery anoles and half as non-recovery. Recovery anoles rested for ~20 min in an isolated 200 ml glass container at 25°C, whereas I picked up non-recovery anoles from the track, anaesthetized them immediately, killed them and dissected the muscles and livers.

The second part of the experiment assessed the effects of alcohol combined with training on activity levels of the catabolic enzyme citrate synthase (CS). I analyzed these results with a two-way factorial ANOVA. When sufficient muscle was removed, I also measured glycogen content to get a baseline level of muscle and liver glycogen, as these groups did not have a final run before euthanasia.

Training

I constructed a 4.88 m track, approximately 0.1 m wide with 0.5m high sides of tempered-hardboard material. I exercised the trained groups to exhaustion nightly for fourteen consecutive days between 5:00pm and 8:30pm by running each along the track,

turning them around to run back at each end. I considered running to one end and back (9.75m) one lap and recorded this distance and their total running time. To encourage them to run, I lightly prodded their tails or the back of their legs with a stick, or waved my hand behind them to simulate a predator approaching. I defined "exhaustion" as the point where I was able to pick up the lizard without resistance.

Alcohol Exposure

During the alcohol exposure treatment, I gave each lizard 3g/kg of ethanol using doses of an 18% ethanol solution (Table 1). This concentration reflects that used by Deckel (1997). I force fed alcohol orally to the treatment groups with a pipette fitted with a clean tip for each individual daily between 8:30 and 11:30 am for 14 days.

Dissections

After their final run, lizards were anesthetized one at a time by placing them in a 250 ml glass bottle on dry ice for 30-60 s before euthenizing them by decapitation with a small razor blade (Greenberg 1992). I removed the quadriceps and illiofibularis muscle from both legs, then the liver, then immediately froze the tissue and the rest of the body in liquid nitrogen. I stored the tissue at -80°C until using it in the biochemical assays described below.

Citrate Synthase Assay

Citrate synthase activity levels were measured using a spectrophotometric assay, as described in Bevier (1995). The assay was conducted at 25ºC and changes in optical

density were measured at 412 nm with a Hitachi UV/VIS-3010 spectrophotometer

equipped with a temperature-controlled cuvette chamber and circulating refrigerated

water bath. Assays were replicated for each sample. For sample preparation, each

muscle was homogenized in a glass-glass homogenizer in a buffer (25mM Hepes, 2 mM

EDTA, 0.5% Triton X-100, pH = 7.0) to break up the muscle cells. The crude

homogenate was centrifuged, and the supernatant transferred to another centrifuge tube. I

broke up the mitochondrial membrane using three freeze-thaw cycles. Samples were

then stored on ice. The assay medium consisted of 100 mM

tris(hydroxymethyl)aminomethane (Tris)-HCl, 2.5 mM EDTA, 0.1 mM 5,5'-dithiobis 2-

nitrobenzoic acid (DTNB), 0.2 mM acetyl-CoA, and 0.5 mM oxaloacetate at a final pH of

8.2 in a total volume of 1.0 mL. This assay provides saturating levels of substrate to

maintain constant enzymatic reaction. Control assays were conducted by placing the

cuvette into the spectrophotometer for two minutes before adding oxaloacetate. The

increase in absorbance was then recorded for 3 min, and the final activity was calculated

as micromoles of product produced per minute per gram tissue.

Glycogen Assay

Glycogen levels were measured using the methods modified from Keppler and

Decker (1974) and Marsh and Dawson (1982). Leg muscle and livers from each lizard

were removed from the freezer, weighed on a Sartorius analytical balance, then

homogenized individually in 0.6 M cold perchloric acid on ice with a glass-glass

homogenizer. 0.2 ml of the crude homogenate was removed (if samples were <0.03 g,

0.1 ml was removed) and treated with amyloglucosidase to digest glycogen to glucose to

determine total glucose content. Supernatant from the remaining homogenate was neutralized and used to determine free glucose content using a glucose diagnostic kit from Sigma (catalog # GAG020). The concentration of glucose in the tissue was measured spectrophotomerically with a Hitachi U-3010 spectrophotometer. Assays were conducted at room temperature (approximately 22ºC) at 450 nm, and two replicates for each tissue sample were analyzed when possible. Values for each replicate were within at most 10% of each other. Mass-specific glycogen content of each tissue sample was calculated by subtracting free glucose from total glucose. The measurement was then converted from glycosyl units (μmol).

Results

Performance

Trained lizards ran farther before exhaustion on the final night of the study period than sedentary lizards (ANOVA, $F = 12.82$, $p = 0.001$, $n = 24$, Figure 1, Tables 2 & 3). Trained lizards without alcohol treatment also ran significantly further than sedentary lizards without alcohol treatment; however, lizards in sedentary and trained alcohol treatment groups did not differ significantly in how far they ran before exhaustion (ANOVA, $F = 15.40$ $p < 0.001$, $n = 12$, Figure 1, Table 3). Trained lizards also had greater average speed in the final performance (ANOVA, $F= 11.75$, $p < .005$, $n = 24$, Tables 2 & 4).

Leg Glycogen Levels

Lizards with a longer recovery time had higher concentrations of glycogen in leg muscles, but the differences were not significant (robust regression, p = 0.598, n = 23, Figure 2, Table 5). Lizards treated with alcohol had significantly higher mean leg glycogen levels than control lizards, and there was a significant interaction between alcohol-treated and trained lizards (robust regression, p < 0.001, Figure 3). Trained, control treatment lizards had significantly lower leg glycogen levels after exhaustive exercise than sedentary control lizards and both trained and sedentary alcohol treatment lizards (robust regression, p < 0.001, n = 12, Figure 4).

Distance the lizard traveled before exhaustion, and ln(distance traveled) were the performance variables most highly correlated with depletion of leg glycogen (robust regression, p = 0.042, Table 6). Finally, liver glycogen levels and leg muscle glycogen levels were positively correlated (robust regression, p = 0.001, Table 6).

Liver Glycogen Levels

Larger lizards had lower mass-specific liver glycogen levels (robust regression p = 0.003, Figure 5). When mass was accounted for, lizards given the alcohol treatment had significantly lower mean liver glycogen than control lizards (robust regression, p = 0.004, n = 35, 34, Table 7). Training did not have a significant effect (robust regression, p = 0.113, n = 35, 34), nor did recovery (robust regression, p= 0.285, n = 24). However, there were significant effects from the interactions between training and recovery, and alcohol and recovery (Figures 6 & 7, Table 7). The resting, control treatment lizards had

significantly less liver glycogen than the lizards dissected immediately after exercise. The resting, alcohol treated lizards had significantly less liver glycogen than every other treatment combination. The trained lizards dissected immediately had significantly less liver glycogen than lizards given 20 min of recovery and resting lizards. Distance traveled before exhaustion also had the most significant relationship of performance variables with liver glycogen levels (Table 8). The further the lizards traveled, the more depleted their liver glycogen stores.

Citrate Synthase

Citrate synthase (CS) activity levels did not differ significantly among treatment groups (Figure 8, Table 9). Lizards treated with alcohol had leg muscles with higher CS activity levels, but body mass and CS activity levels were highly correlated (regression, $p = 0.0003$), as were alcohol and body mass (robust regression, $p < 0.001$).

Discussion

Trained lizards exhibited improved exercise performance, as reflected in increased average $(speed)^2$ and greater ln(distance) compared to untrained lizards. This positive change in performance contradicts results of other studies, which demonstrated decreased speed and endurance in trained individuals because of pathological effects of training (e.g., Garland et al. 1987). These results show that lizards may indeed have the capacity to improve performance, but perhaps only after my shorter training regimes.

The mechanism of improvement remains to be tested and any changes are probably anaerobically associated. Citrate synthase activity levels have been shown to be a good indicator of aerobic activity capacity (Bevier 1995), and as activity levels of citrate synthase in leg skeletal muscle did not significantly differ between treatment groups, improvement in performance is not likely a result of enhanced aerobic pathways.

Training effects on distance (ln(distance)) traveled before exhaustion varied among individuals in alcohol and control groups. Trained lizards given alcohol did not run any further than sedentary lizards given alcohol, indicating that alcohol treatment negated positive effects of training on exercise performance. The average distance run was higher in alcohol treated lizards than the sedentary control group, but alcohol treated lizards were larger, on average than those in the control groups, and this may account for the difference.

The effects of training and alcohol exposure on the rate of leg glycogen resynthesis during recovery cannot be determined from this study because the leg glycogen concentrations immediately after exercise and after a 20 min recovery were not significantly different. A larger lizard (~50g), *Dipsosaurus dorsalis*, was fully recovered from exhaustive exercise after approximately two hours (Gleeson and Dalessio 1989). Leg glycogen levels in the same lizard increased during recovery, and were significantly greater after 30 - 60 min. of recovery than immediately after exercise (Gleeson and Dalessio 1989). Bennett and Licht (1972) reported a 90 min recovery period in green anoles (*Anolis carolinensis*), which are comparable to brown anoles. If both species have similar rates of recovery, leg glycogen concentration would likely be significantly higher after 25 - 45 min of recovery, as opposed to the 20 min period used in this study. Anoles

in this study recovered at ~26ºC, which was comparable with temperatures during recovery in *A. carolinensis* (Bennett and Licht 1972). Therefore temperature did not likely influence recovery.

Gleeson (1985) also documented decreased capacity for lactate gluconeogenesis in *Dipsosaurus dorsalis* caught in December compared to those caught in August. This reflects a greater capacity during the lizard's more active season. It is possible that anoles could have similar flexibility. Lizards in the present study were collected in October and December, and may consequently have had diminished skeletal muscle lactate gluconeogenesis to begin with given that they may go into a more inactive state during cooler months.

Although the influence of alcohol and training on recovery could not be conclusively assessed in this study, these two treatments still had interesting effects on skeletal muscle and liver glycogen concentrations. First, alcohol-treated lizards had significantly higher leg glycogen concentrations after exercise than control lizards, even after the 20 min recovery period. This could either indicate that initial glycogen levels were lower in control individuals, or that control lizards were able to use their energy stores more efficiently. Evidence supports the latter explanation: the significant inverse relationship between distance traveled before exhaustion and leg glycogen concentrations shows that lizards that travel a greater distance use more glycogen. The interaction between alcohol and training groups was also significant because the trained, control lizards had significantly lower leg glycogen concentrations than the three other treatment combinations. Trained, control anoles also ran the furthest before exhaustion and, as distance traveled and leg glycogen levels are related, this explains why their leg muscle

glycogen concentrations were lowest. The recovery period was not long enough for a lizard from any treatment group to completely replace its glycogen stores, therefore leg glycogen levels reflect those from immediately after exercise.

Distance traveled before exhaustion was also inversely related to liver glycogen stores, which were in turn positively related to leg glycogen. These relationships suggest that the liver glycogen may indeed have had a role during exercise, and perhaps the muscles were not operating in a system independent of liver glycogen modulation. Larger lizards also had lower mass-specific liver glycogen concentrations, indicating that smaller lizards may be more dependent on these liver glycogen stores.

During recovery, liver glycogenolysis contributed to elevated blood glucose levels in *Dipsosaurus dorsalis*, but it is unclear whether or not this glucose was taken up by the muscles (Gleeson and Delassio 1989). When lactate and glucose with isotopic labeled carbons were injected into the recovering lizards, carbons from injected blood lactate were not found in the liver, and blood glucose had minimal contribution to leg glycogen resynthesis (Gleeson and Delassio 1989). Therefore, unlike in mammals, lactate is not being cycled through the liver in this iguanid species.

In this study, the interaction between recovery and training does suggest possible lactate reuptake by the liver. Trained lizards had significantly lower liver glycogen both immediately and 20 min after exercise than sedentary lizards, and the lizards sacrificed immediately also had lower levels than those sacrificed after a recovery period. As the trained lizards ran further and distance was related to liver glycogen, it appears that they had low glycogen levels immediately after exercise, which increased during the recovery. Glucose concentrations after exercise were presumably low, making blood lactate the

likely substrate for hepatic gluconeogenesis. The interaction between alcohol groups and recovery showed that at rest, alcohol groups had significantly lower mean liver glycogen concentration that control groups. This is consistent with observations in mammals of increased glycogenolysis and decreased gluconeogenesis resulting in low liver glycogen (Heikkonen et al 1998).

Conclusion

Contrary to previous research, training may improve exercise performance in a lizard, the brown anole. A brief, two-week training period resulted in increased performance speed and distance before exhaustion in trained lizards. Trained lizards were also able to more effectively use leg glycogen stores, however each of these improvements were not found in lizards treated with alcohol. Liver glycogen concentrations were also lower in alcohol-treated lizards, and patterns of liver glycogen concentrations during recovery indicate some hepatic lactate gluconeogenesis.

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Table 1. Alcohol dosages of an 18% ethanol solution for ranges of anole mass.

Mass Anole (g)	Dose (in μl)	Mass Anole (g)	Dose (in μl)	Mass Anole (g)	Dose (in μl)	Mass Anole (g)	Dose (in μl)
0.50-0.75	1.9	2.25-2.5	8.6	4.0-4.25	15.2	5.75-6.0	21.9
0.75-1.0	2.9	2.5-2.75	9.5	4.25-4.5	16.2	6.0-6.25	22.8
1.0-1.25	3.8	275-3.0	10.5	4.5-4.75	17.1	6.25-6.5	23.8
1.25-.1.5	4.8	3.0-3.25	11.4	4.75-5.0	18.1	6.5-6.75	24.7
1.5-1.75	5.7	3.25-3.5	12.4	5.0-5.25	19.0	6.5-6.75	24.7
1.75-2.0	6.7	3.5-3.75	13.3	5.25-5.5	20.0	6.75-7.0	25.7
2.0-2.25	7.6	3.75-4.0	14.3	5.5-5.75	20.9	7.0-7.25	26.6

Table 2. Mean values (± SE) for performance variables in sedentary and trained lizards.

Performance Variable	Sedentary	Trained
ln(Distance) (body lengths)	4.57684± .0801	4.92734± .0645
Speed$^{1/2}$ (body lengths/s)$^{1/2}$	0.64100± .0228	0.75301± .01862

Table 3. Results of ANOVA of treatment and treatment combination effects on ln(performance distance).

Source	SS	df	MS	F	Prob > F
Alcohol	0.042	1	0.042	0.42	0.523
Training	1.301	1	1.301	12.82	0.001
Recovery	0.138	1	0.138	1.36	0.250
Alcohol x Training	1.562	1	1.562	15.40	0.000
Alcohol x Recovery	0.048	1	0.048	0.47	0.496
Training x Recovery	0.054	1	0.054	0.53	0.471
Residual	0.456	40	0.011		
Total	0.599	46	0.013		

Table 4. Results of ANOVA of treatment and treatment combination effects on speed[2].

Source	SS	df	MS	F	Prob > F
Alcohol	0.00385	1	0.00385	0.34	0.5647
Training	0.13398	1	0.13398	11.75	0.0014
Recovery	0.00179	1	0.00179	0.16	0.6937
Alcohol x Training	0.00081	1	0.00081	0.07	0.7913
Alcohol x Recovery	0.00126	1	0.00126	0.11	0.7417
Training x Recovery	0.00063	1	0.00063	0.06	0.8148
Residual	0.46415	40	0.01140		
Total	0.59599	46	0.01302		

Table 5. Results summary of robust regression analysis for the effects of training, alcohol treatments, and treatment combinations on leg glycogen levels.

Source	t	p-value
Alcohol	-3.910	0.000
Training	0.980	0.344
Recovery	-0.530	0.598
Alcohol x Training	4.050	0.000
Alcohol x Recovery	-0.190	0.850
Training x Recovery	0.870	0.388

Table 6. Summary of individual robust regression analysis between leg glycogen levels and performance variables, mass, and liver glycogen.

Source	t	p-value
Time	-1.58	0.122
Distance	-1.95	0.057
ln(distance)	-2.09	0.042
Speed	-0.5	0.618
(Speed)^.5	-0.55	0.582
Mass (g)	-1.5	0.142
Liver Glycogen	3.54	0.001

Table 7. Results summary of robust regression analysis for the effects of training, alcohol treatments, and treatment combinations on liver glycogen levels.

Source	t	p-value
Body Mass	-3.14	0.003
Alcohol	3.03	0.004
Training	1.61	0.113
Recovery	2.25	0.1147
Alcohol x Training	-0.82	0.415
Alcohol x Recovery	-3.18	0.0025
Training x Recovery	2.21	0.038

Table 8. Summary of individual robust regression analysis between performance variables and liver glycogen.

Source	t	p-value
Distance	-1.95	0.06
ln(distance)	-1.63	0.113
Speed	-0.71	0.483
Time	-1.16	.256

Table 9. Results of ANOVA on treatment and treatment combination effects on (Citrate Synthase Activity)^2.

Source	SS	df	MS	F	Prob > F
Alcohol	239.5150	1	239.5150	2.52	0.128
Training	1.0131	1	1.0131	0.01	0.9188
Alcohol x Training	9.7946	1	9.7946	0.1	0.7514
Residual	1899.5431	20	94.9772		
Total	2154.4693	23	93.6726		
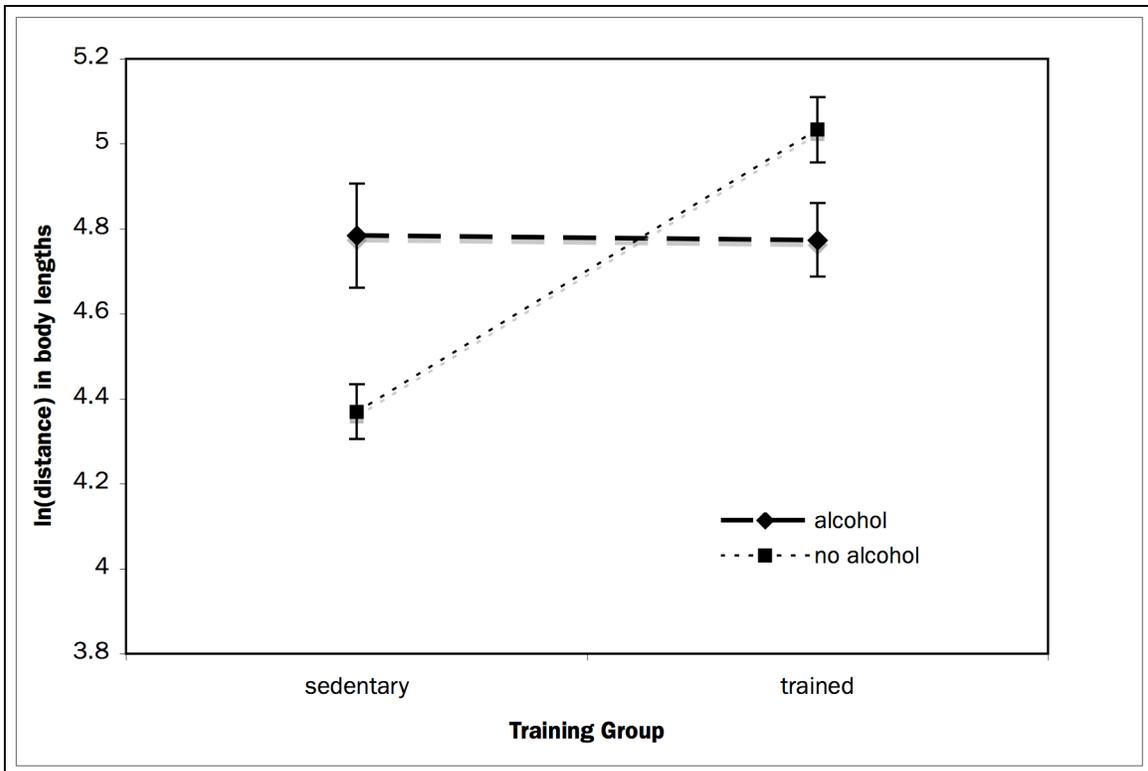
Figure 1. Effects of alcohol and training on performance (ln(distance) run before exhaustion) in lizards (ANOVA, p < 0.0005, n = 12). The interaction between variables is significant and shows that trained control groups ran further than trained control groups, but there was no difference in distance run between alcohol-treated trained and sedentary anoles.
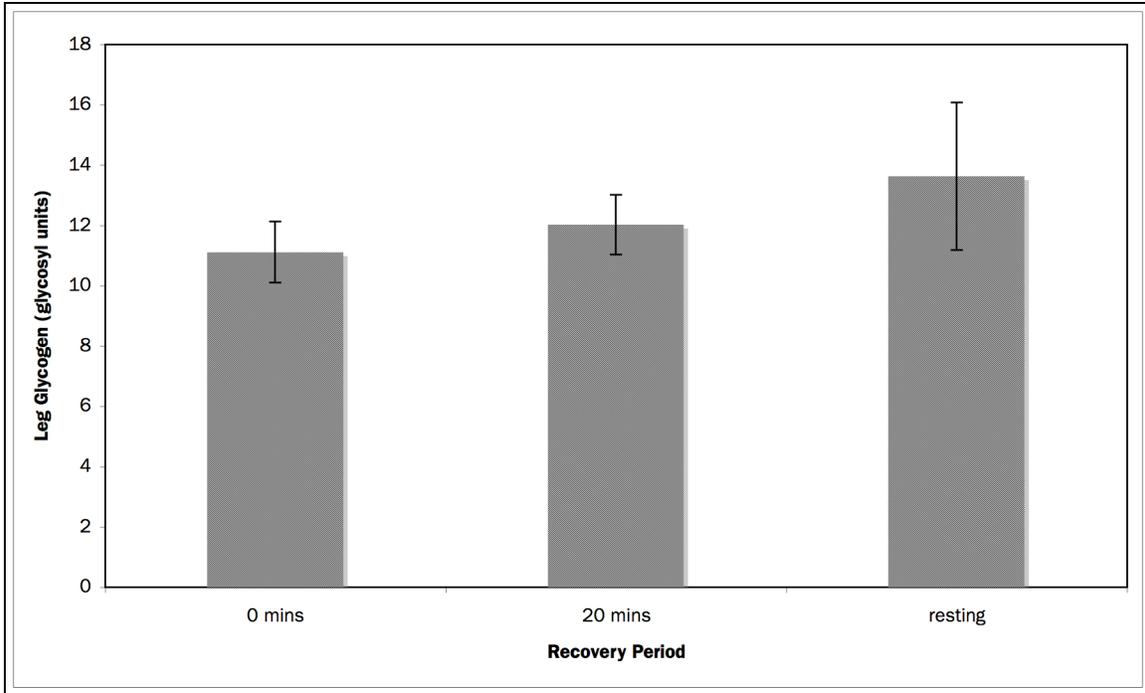
Figure 2. Glycogen concentration in leg muscles of lizards dissected immediately after exercise, twenty minutes after exercise, and at rest (Robust Regression, $p = 0.598$, $n = 23$, 23, 6). Bars indicate $\pm$ 1SE.
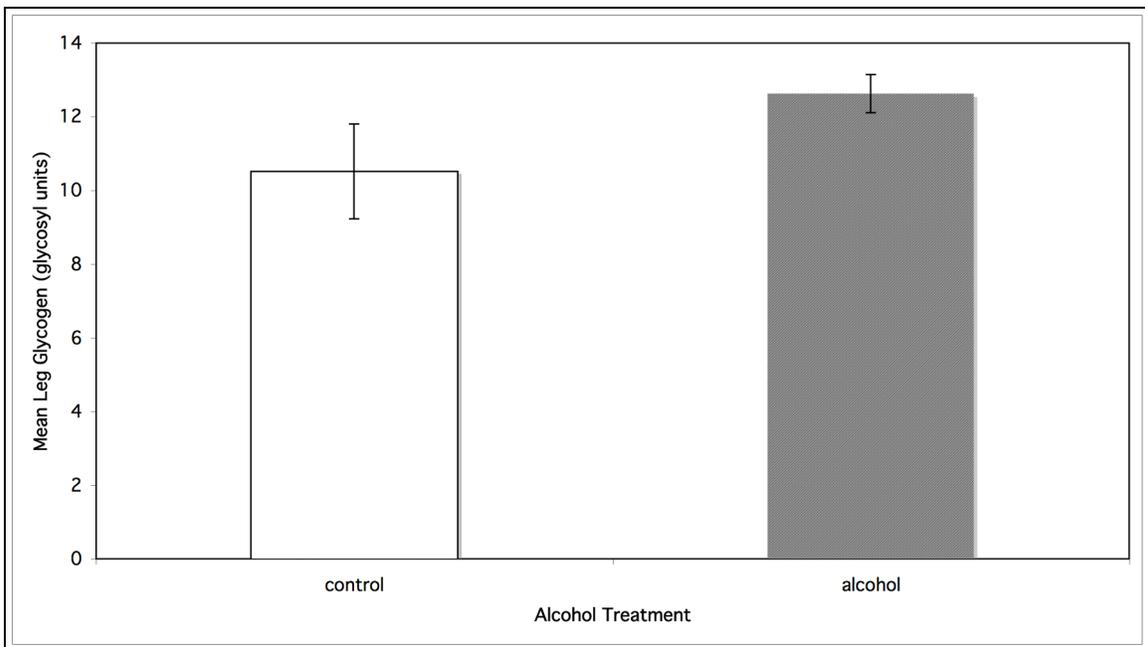
Figure 3. Mean leg glycogen levels in alcohol treated lizards and control lizards (robust regression, $p < 0.001$, $n = 23$). Bars indicate $\pm$ 1SE.
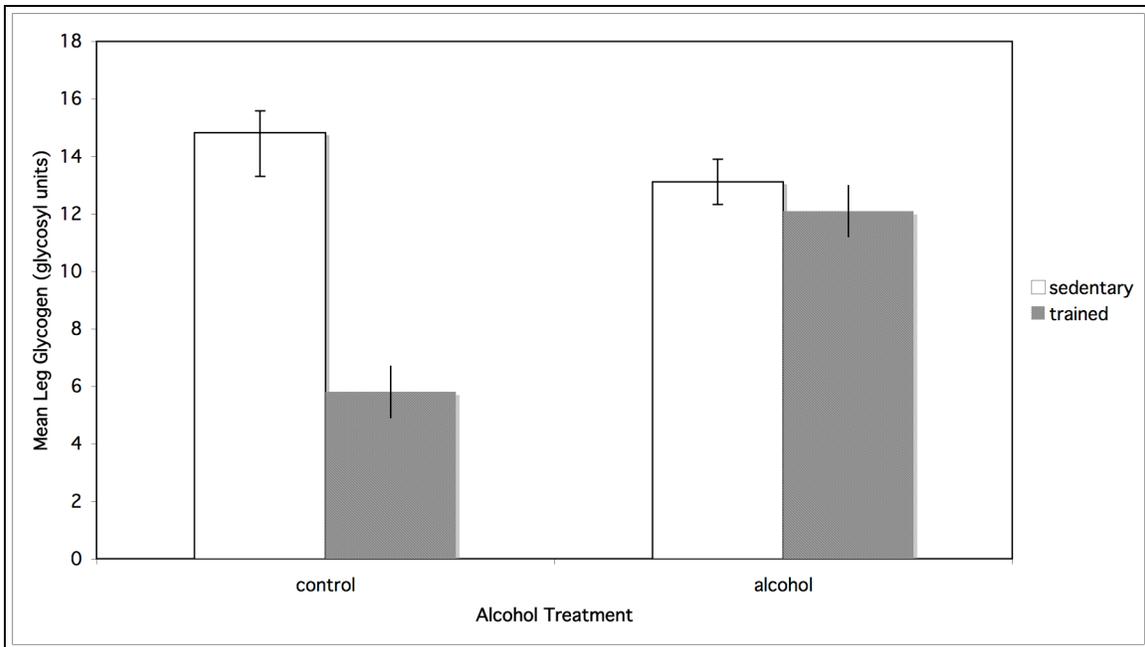
Figure 4. Effect of alcohol treatment and training on mean leg glycogen levels. The interaction between treatments is significant where trained, control anoles had lower leg glycogen concentrations that the remaining three treatment combinations (robust regression, $p < 0.001$, $n = 12$).   Bars indicate ± 1SE.
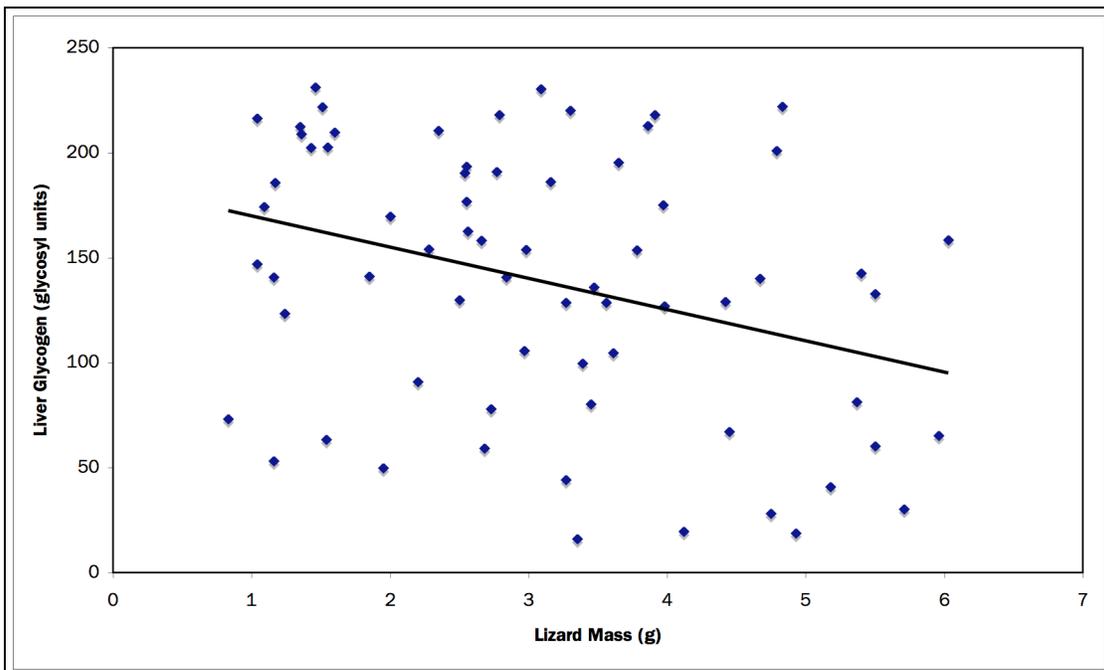

Figure 5. Relationship between mass-specific liver glycogen levels and lizard body mass (robust regression, $p = 0.003$, $R^2 = .1113$).
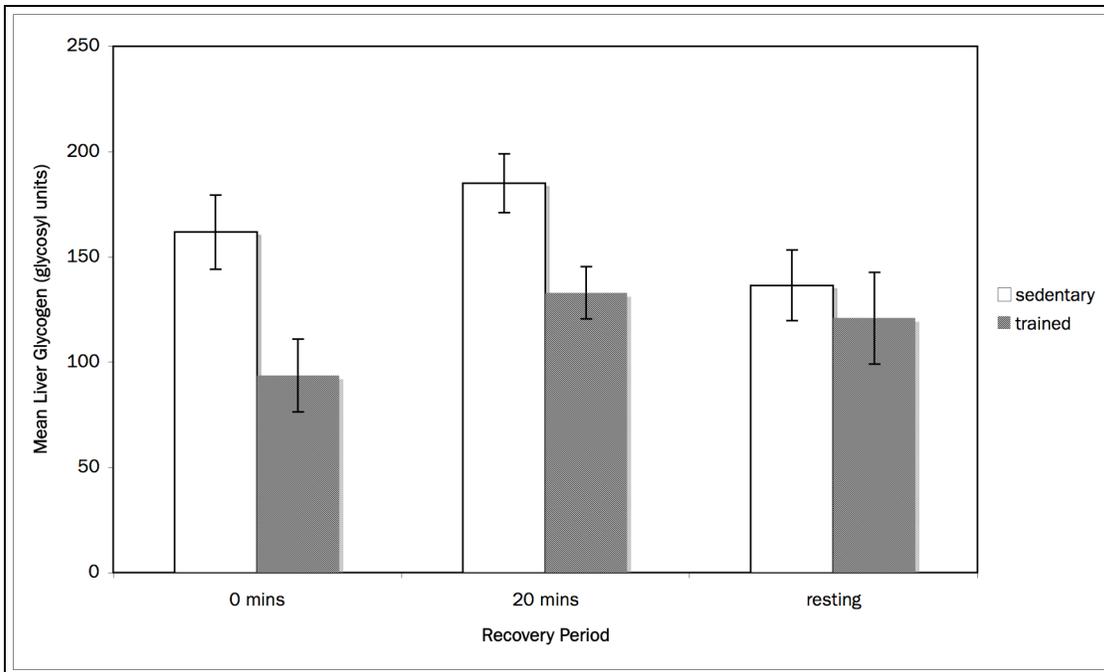
Figure 6. Mean liver glycogen levels for sedentary and trained lizards after three different periods of recovery from exercise (robust regression, p = 0.038, n = 12). Bars indicate ± 1SE. Trained lizards have significantly lower liver glycogen concentrations immediately after exercise and after a recovery period, but not at rest.
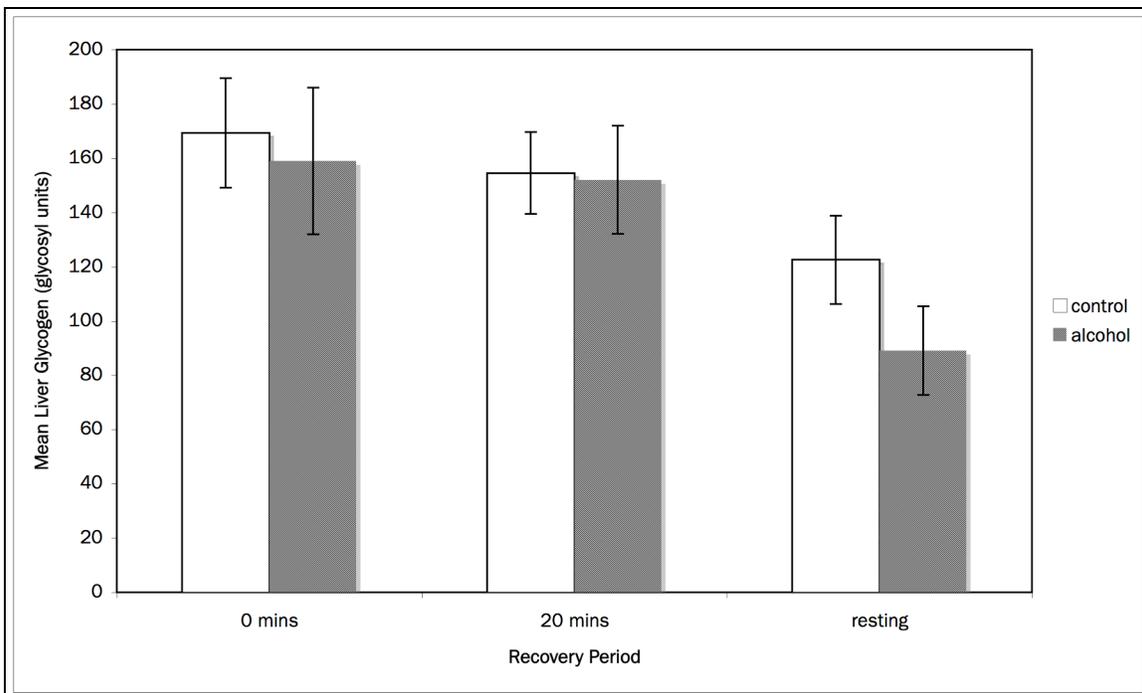

Figure 7. Mean liver glycogen levels for alcohol treated lizards and control lizards after three periods of recovery after exercise (robust regression, p = .0025, n = 12). Bars indicate ± 1SE. This interaction of treatments shows that alcohol-treated lizards have lower liver glycogen concentrations at rest.
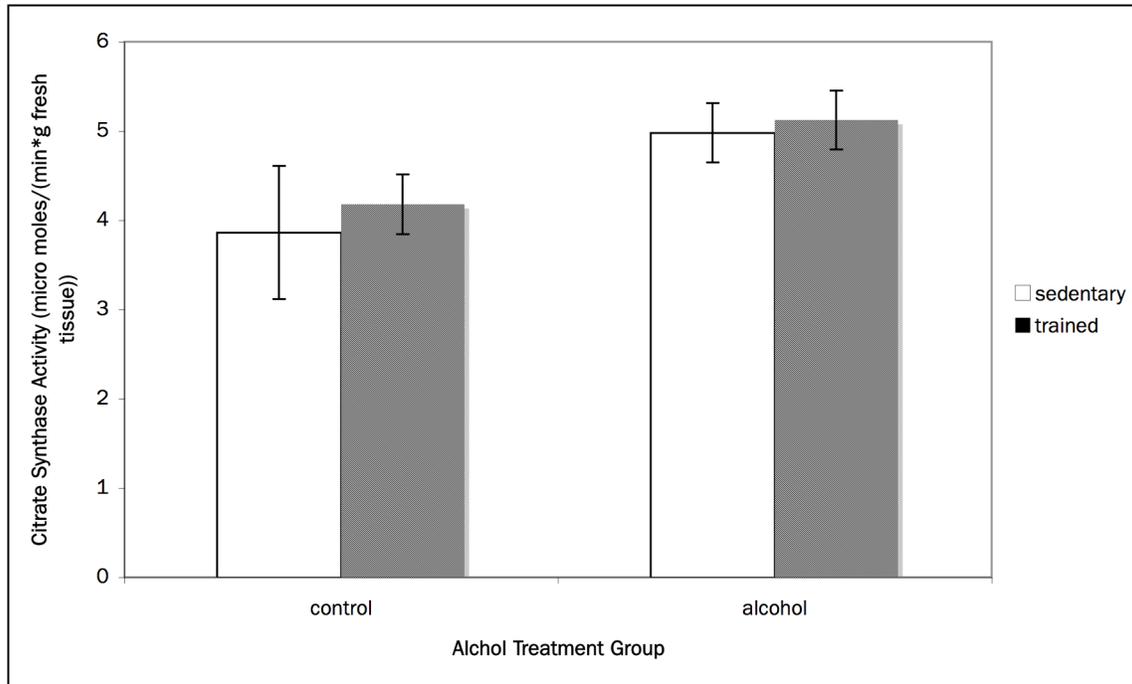
Figure 8. Average activity levels of citrate synthase in lizard leg skeletal muscle of trained and alcohol treated groups (n = 6). Bars indicate ± 1SE.