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The Effects of Exogenous Insulin and Exercise on Glucose and Lactate Metabolism in the Brown Anole, Anolis sagrei

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The Effects of Exogenous Insulin and Exercise on Glucose and Lactate Metabolism in the Brown Anole, *Anolis sagrei*

By Kristen Marie Hitchcox

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The Effects of Exogenous Insulin and Exercise on Glucose and Lactate Metabolism in the Brown Anole, *Anolis sagrei*

By Kristen Marie Hitchcox

Approved:

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Mentor, Dr. Catherine Bevier, Associate Professor of Biology

_____Date

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Reader, Dr. Julie Millard, Dorros Professor of Chemistry

_____Date

DEDICATION

I dedicate this project to my brother and great friend, Douglas Paul Hitchcox. The purpose behind this research is to learn more about metabolic processes related to diabetes mellitus. I hope it may help determine new treatment methods. Doug is my inspiration and my motivation. This research project is the first of many that will be carried out with Doug in my heart.

VITA

Kristen Marie Hitchcox was born on May 3, 1987 in Portland, Maine, to Paul and Susan Hitchcox. Paul is an actuary and received his Bachelor of Arts from the University of Southern Maine in Mathematics. Susan is an occupational therapist and received her Bachelor of Science from Tufts University in Occupational Therapy. Kristen also has a younger brother named Douglas who attends the University of Maine at Orono. Paul, Susan, Kristen and Douglas live together in Hollis, Maine.

Kristen attended Bonny Eagle High School in Standish, Maine. She took general college prep classes and was inspired by her chemistry teacher to pursue an undergraduate degree in chemistry. After graduating in 2005, she attended Colby College in Waterville, Maine. At Colby, Kristen was a member of the Chemistry Club, Student Health on Campus, and Colby Cares About Kids, volunteered for Admissions, and worked in the Chemistry department as a lab assistant and a tutor. Kristen will graduate with Chemistry Honors on May 24, 2009, with a degree in Chemistry: Biochemistry and American Chemical Society certification.

Kristen plans to attend graduate school in the fall of 2010. She aspires to do biochemistry research related to the prevention and treatment of type I diabetes mellitus.

ABSTRACT

Blood glucose homeostasis is tightly regulated in mammals. Insulin and exercise both stimulate glucose uptake into muscle cells via the GLUT4 transporter protein by independent pathways. Insulin triggers a tyrosine kinase pathway, but the mechanism stimulated by exercise is unknown. Discovering the mechanism may provide new therapeutic techniques for people with diabetes mellitus. While mammals have been studied extensively, research on a different model may elucidate aspects of this lesser known pathway. Reptiles rapidly deplete glycogen stores during bursts of activity and produce lactate as a byproduct of anaerobic metabolism. Lactate undergoes gluconeogenesis within muscle tissue, rather than liver tissue, to quickly replenish glycogen stores. Lactate is a preferred substrate for gluconeogenesis; thus, glucose uptake is inhibited. Since exercise has a significantly different effect on metabolism in reptiles, a reptilian model may provide novel information as to how exercise stimulates glucose uptake in mammals. *Anolis sagrei* were treated with combinations of exogenous insulin and exercise. Blood glucose, blood lactate, muscle glycogen, liver glycogen and muscle GLUT4 were measured. There was no significant treatment effect on levels of post-exercise blood glucose, however, levels of blood lactate increased significantly following exercise. Muscle glycogen was generally lower following exercise, though liver glycogen was not significantly different. GLUT4 was not detected. Finally, there was no difference in the level of any monitored metabolite following insulin injection. It appears that (1) gluconeogenesis occurs in muscle rather than liver tissue; (2) muscle contractions do not stimulate glucose uptake; (3) insulin may not affect glucose metabolism; (4) an isoform of the GLUT4 transporter protein may not exist. Future studies should compare the relative activities of effectors in reptiles and mammals following exercise to help elucidate the mechanism of the contraction-stimulated glucose uptake pathway in mammals.

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INTRODUCTION

Metabolic pathways regulate the production and storage of energy in organisms. Heterotrophic organisms, such as mammals and reptiles, depend on organic sources in the environment to fuel metabolism, including proteins, fats, and sugars. These nutrients are consumed to gain energy through amino acid, lipid, and carbohydrate metabolism, respectively. Complex metabolic processes work together to fuel an organism's energetic needs to live, grow and reproduce.

Arguably the most important carbohydrate of carbohydrate metabolism is the six-carbon monosaccharide, glucose. This simple sugar has been called "the nearly universal fuel and building block in modern organisms." [1] Glucose is formed by the breakdown of sugars, such as lactose and sucrose, which are common in most animal diets. It is also formed by the phosphorolysis of glycogen, a polysaccharide of stored glucose monomers. Glucose is an energy-rich nutrient; the complete combustion, $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$, yields 2,840 kJ/mol. Cells liberate the potential energy from glucose in a series of reactions to yield energy to synthesize adenosine triphosphate (ATP). Glucose fuels immediate energetic needs of the muscles and provides the majority source of energy for the human brain, nervous system, erythrocytes, testes, renal medulla and embryonic tissue.[1]

The breakdown of sugars by carbohydrate catabolism begins with the pathway of glycolysis. Glycolysis is a 10-step, two-phase series of priming, cleaving, oxidation and phosphorylation reactions that occur in the cytosol and that rely on specific enzymes to catalyze each step. Oxidation reactions require oxidizing agents such as NAD^+. Oxidizing agents are regenerated under aerobic conditions by the electron transport chain when electrons stored in NADH are ultimately transferred to O_2**.** Oxidizing agents are regenerated under anaerobic

conditions by the reduction of pyruvate to lactate. Glycolysis ultimately produces 2 moles of pyruvate, 2 moles of ATP and 1 mole of NADH for each mole of glucose catabolized. The overall standard free-energy change is -85 kJ/mol, making glycolysis a favorable, spontaneous, and irreversible reaction when all enzymes and substrates are available.[1]

Pyruvate has three catabolic fates. Under aerobic conditions it enters the citric acid cycle along with compounds derived from lipid and amino acid catabolism. The citric acid cycle oxidizes its carbon intermediates to CO_2, simultaneously yielding ATP. Electrons lost during the oxidation reactions are transferred to oxidizing agents FAD and NAD^+. Similar to glycolysis, $FADH_2$ and NADH are regenerated through oxidation by O_2 in the electron transport chain. This overall process is called cellular respiration.[1]

However, anaerobic conditions exist when an organism is not consuming enough oxygen to fuel aerobic metabolism. This occurs in vigorously contracting muscle, where high levels of ATP are needed to fuel contractions. High amounts of oxygen are also needed to regenerate the oxidizing agents needed to fuel glycolysis and the citric acid cycle. Oxygen consumption and release is limited by rates of respiration and availability of hemoglobin and myoglobin. These factors are not able to compensate for the increased oxygen needed under times of stress. Oxidizing agents to fuel glycolysis and the citric cycle are limited and insufficient in the absence of the terminal electron acceptor, oxygen, in the electron transport chain. Pyruvate must therefore have an alternative anaerobic fate.[1]

In mammals and reptiles under anaerobic metabolic conditions, pyruvate produced in hypoxic muscle tissue undergoes fermentation to produce lactate. This reaction is catalyzed by lactate dehydrogenase and is highly exergonic, with a change in free energy of -25.1 kJ/mol. The reduction of two pyruvate molecules uses two pairs of electrons from NADH to form two lactate

molecules, therefore regenerating two NAD^+ oxidizing agents. After lactate fermentation, there is no net loss in reducing agents and glycolysis is no longer inhibited. The citric acid cycle, however, still does not have sufficient oxidizing agents to funnel pyruvate through it. The majority of energy produced by glucose catabolism occurs through the glycolytic pathway.[1]

According to Le Châtelier's principle, high concentrations of reactants will drive a reaction forward. Therefore, to drive muscle glycolysis forward to produce more ATP during vigorous exercise, there needs to be an increase of glucose in the muscle tissue. Other times an organism would need an increased amount of glucose in its tissues are between meals or during fasts when glycogen stores are depleted. Specifically after the anaerobic production of lactate, the process of transporting glucose into muscles to fuel glycolysis and replenish glycogen stores is called the Cori Cycle.

The Cori Cycle begins by the transport of lactate across skeletal muscle membranes to the blood. Lactate (pK_a= 3.86) is primarily dissociated into an anion and a proton at physiological pH. In mammals, the lactate anion is most commonly transported by a monocarboxylate transporter, which acts as a lactate/proton symporter.[2] Lactate in the blood is then taken into the liver to undergo gluconeogenesis. This pathway shares seven of the ten steps in glycolysis, catalyzed by the same enzymes but run in the reverse direction. The remaining three steps are the committing, irreversible steps of glycolysis and cannot be run in reverse. These reactions are "bypassed" in gluconeogenesis by a separate set of enzymes. For example, the final reaction that generates glucose is catalyzed by glucose 6-phosphatase. This enzyme is only found in mammals on the lumenal side of the endoplasmic reticulum of hepatocytes and renal cells. The enzyme is tissue specific; thus, gluconeogenesis is only seen in the liver and kidney of mammals.[1]

Gluconeogenesis is a critical pathway when anaerobic conditions deplete glycogen stores in muscle tissue. It is also important to regenerate NAD^+ oxidizing agents. However, gluconeogenesis is energetically expensive and consumes 4 moles of ATP for every 2 moles of pyruvate. The three irreversible reactions that bypass the irreversible reactions of glycolysis are therefore necessary to ensure the overall pathway lies toward the production of glucose. The total change in Gibb's Free Energy is -16 kJ/mol, making gluconeogenesis an irreversible process.[1]

Newly synthesized glucose is then transported into the bloodstream and returned to muscles to replenish their glycogen stores. This final step links the Cori Cycle together, as seen in Figure 1.[1] In summary, hypoxic conditions such as vigorous exercise result in depletion of glycogen stores, production of lactate by fermentation, and then resynthesis of glucose by gluconeogenesis in the liver for energy. These metabolic processes have been extensively studied in humans, rats, and mice. In all of these mammalian models, exercise has been shown to increase glucose transport in muscle tissues.[3]

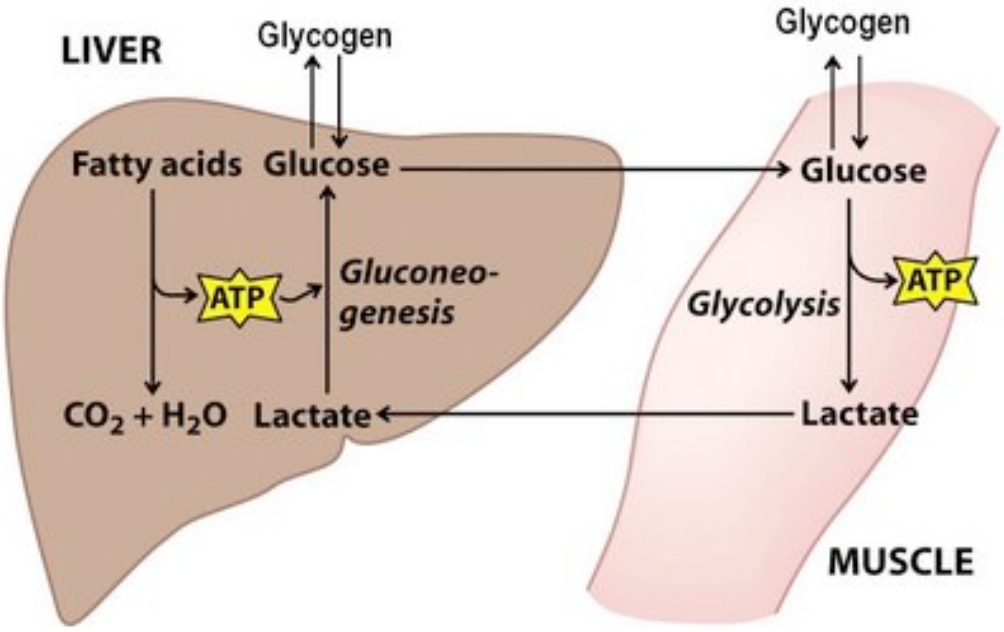

Figure 1. The Cori Cycle. Glucose is anaerobically metabolized to lactate in muscle, then transported to the liver to undergo gluconeogenesis and produce glucose.[1]

Glucose is a polar molecule; it requires a transporter protein to cross hydrophobic plasma membranes. In specific tissues of mammalian intestine and kidney, there are secondary active Na^+/glucose symporters. In all other mammalian tissues, there are facilitative uniporters. These energy-independent glucose carriers make up the GLUT protein family. All GLUT proteins are made of 12 transmembrane segments and transport glucose using heterologous expression systems. To date, six mammalian GLUT isoforms have been identified. They exhibit tissue-specific distribution and transport glucose with different efficiencies and kinetics. Different functions of the proteins range from basal glucose transport to transport across the blood brain barrier.[4]

The role of the GLUT2 protein is to transport glucose from the bloodstream into β cells of the pancreas. Glucose then immediately undergoes glycolysis, the citric acid cycle, and oxidative phosphorylation to produce ATP. Under hyperglycemic conditions, there is an elevated level of glucose circulating in the blood. This occurs after eating a carbohydrate-rich meal[1] or during physiological stress such as inflammation.[5] Increased blood glucose drives the production of more ATP in β cells, which causes ATP-gated K^+ channels on the β cell membrane to close. The increase in K^+ within the cytosol depolarizes the membrane and causes voltage-sensitive Ca^{2+} channels to open. The influx of Ca^{2+} triggers insulin release by exocytosis.[1]

Insulin is a peptide hormone found in pancreatic cells. It is first synthesized in the nucleus as preproinsulin, with a signal sequence at its amino terminus that directs its passage into secretory vesicles in β cells. The signal sequence is degradaded by proteases to produce proinsulin. When levels of Ca^{2+} rise rapidly in β cells due to elevated blood glucose, a peptide chain on proinsulin is cleaved to produce insulin. In its active form, the hormone is made of two polypeptide chains connected by two disulfide bonds.[1]

Once secreted, insulin is transported through the bloodstream and binds to insulin receptors found in almost all types of tissue.[6] The insulin receptor autophosphorylates itself and causes a kinase cascade. One downstream effect of the insulin pathway is the phosphorylation and activation of protein kinase B (PKB). In muscle tissue, PKB stimulates intracellular vesicles containing GLUT4 proteins to fuse with the plasma membrane. This causes an increased concentration of GLUT4 in the plasma membrane. GLUT4 transports glucose into the cell so that blood glucose levels return to their normal level, 60-90 mg/100mL.[1] Even though hyperglycemia is present throughout the whole bloodstream, GLUT4 is found exclusively in adipocytes and myocytes. These tissues are insulin-sensitive and regulate blood glucose levels for the whole-body. Under basal conditions, skeletal muscle accounts for 20% of glucose disposal; under hyperinsulinemia, 75-90%.[4] Insulin-stimulated glucose transport is therefore critical for maintaining glucose homeostasis.

Whereas the flux of glucose in the pancreas through GLUT2 transporters is dependent on the concentration of glucose in the blood, the flux of glucose in muscle cells is dependent on the concentration of GLUT4 transporters on the plasma membrane. Glucose transport is therefore the rate-limiting step for glucose uptake into muscles. Similarly, glucose uptake is the rate-limiting step for glycolysis.[4]

It is evident that both insulin and exercise stimulate glucose transport in mammals. Insulin activates GLUT4 vesicles through a signaling pathway involving PKB. Exercise triggers the Cori Cycle to replenish depleted muscle glycogen stores. Exercise also activates GLUT4 vesicles, but the signaling pathway is unknown. It is currently thought that multiple pathways may be involved. These pathways may involve the enzymes AMP-activated protein kinase (AMPK), calcium-activated kinases, nitric oxide, bradykinin, and AS160.[7] Although the pathway

is unknown, increased GLUT4 due to exercise has been shown in numerous animals including humans, rats[3] and frogs[8].

Muscle contraction has also been shown to increase the rates of transcription and translation of GLUT4. In one study, muscle nuclei from rats were isolated after a single bout of exercise and after training; GLUT4 transcription was analyzed by nuclear run-on analysis and was seen to increase 1.8-fold after 3 hours.[3] In another case, polysome-associated GLUT4 mRNA increased immediately following exercise, supporting that translation of GLUT4 is also stimulated by exercise.[3] The mechanism for increased transcription and translation remains unknown. Evidence has suggested that high-energy phosphates that decline during exercise may increase GLUT4 expression. Other evidence suggests that AMP-activated protein kinase, which is activated by exercise, may activate MEF-2 and GEF. These transcription factors are known to be important for GLUT4 expression. NRF-1 is another transcription factor activated by AMPK that may play a role. An increase in calcium released during muscle contraction may also be related to GLUT4 activation.[3]

Interestingly, it is well known that the exercise-stimulated glucose transport pathway is independent of the insulin-stimulated pathway, as illustrated in Figure 2. Many studies have shown that muscle contraction can stimulate glucose transport even in the absence of insulin. Other studies show that the effects of muscle contraction and insulin are additive.[8] Mammalian models used in these studies have provided inconclusive evidence regarding the independence of the two pathways. Investigating the pathways in a non-mammalian model may provide new information or ideas to help solve the puzzle in mammals.

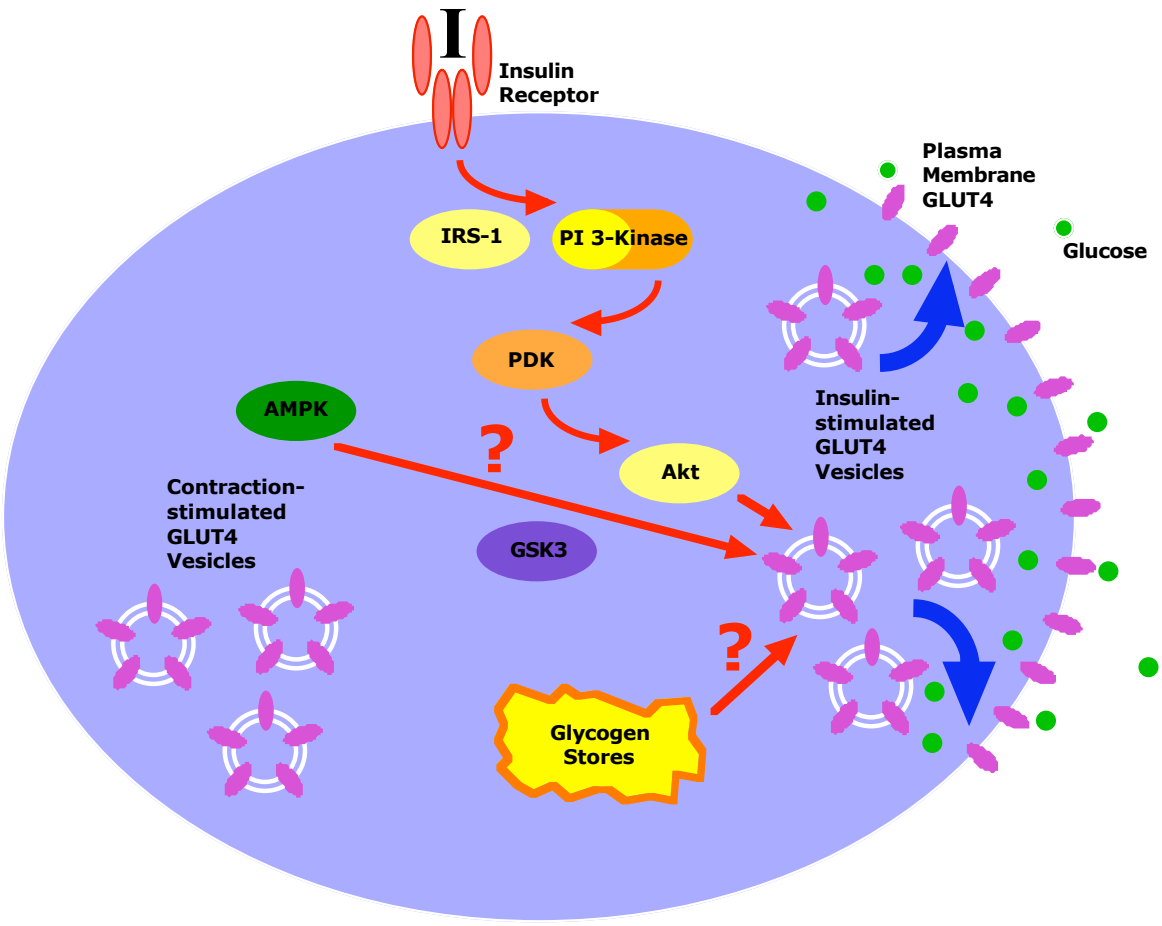

Figure 2. GLUT4 pathways. Muscle contraction and insulin stimulate GLUT4 vesicles through independent pathways; the mechanism of the contraction-stimulated pathway is unknown.

In particular, lizards may provide such a model. Glucose metabolism in several different lizard species has been well studied to discover unusual gluconeogenic pathways. Reptiles and amphibians rely more heavily on anaerobic glycolysis for energy during exercise or hypoxic conditions than mammals do. Therefore, lizards provide an interesting opportunity to study glucose metabolism.

Lizard species that are “sit and wait” predators are the most frequent subjects of studies on exercise physiology. These lizards are at rest most of the time, and exhibit low metabolic

rates. When a mating, feeding, or escape opportunity arises, metabolic costs become secondary[9]. Lizards quickly expend large amounts of energy in bursts of vigorous activity. Energy to fuel these high locomotor costs is produced by breaking down muscle glycogen stores into glucose monomers, which are metabolized to produce ATP.[10]

Because lizards typically exhibit low metabolic rates, a sudden increase in glucose catabolism cause rapid depletion of oxygen levels. Without substrates for aerobic metabolism, lizards switch to anaerobic metabolism. Lactate builds up quickly in the muscles to reach concentrations approximately two fold higher than those seen in mammals and some amphibians.[16] For example, the desert iguana, *Dipsosaurus dorsalis,* has an average resting lactate concentration of 0.25mg lactate/g body weight; post-activity the lactate concentrations rise to 1.5 mg/g.[10] On average, small lizards accumulate 8.5 mmol lactate/kg body weight during the first 30 seconds of exercise.[11] Lactate is found throughout the whole body and can have negative effects on blood pH and oxygen transport in lizards.[12]

High concentrations of lactate lead to long recovery periods in lizards.[9,10] Some lizards have been shown to have low concentrations of myoglobin. In addition, high body temperature associated with vigorous activity causes a decrease in blood oxygen affinity and capacity. These synergistic factors contribute to the long recovery time for oxygen consumption to return to resting levels.[12] However, once the basal respiration rate is reached, lactate levels remain elevated for an additional length of time.[9,10,12] While lactate removal in mammals is often complete within 30 minutes, lactate removal in reptiles such as *Dipsosaurus dorsalis* can take over two hours.[13]

Lactate removal in mammals is not only faster but is also driven by different biochemical needs than in reptiles. Mammals remove lactate through oxidative pathways to return their blood

to its natural acid-base balance and prevent muscle fatigue. Lactate can be transported by a "lactate shuttle" to oxidative fibers such as cardiac muscle to produce CO_2 and H_2O;[14] lactate can also be transported from the muscle to the liver through the Cori Cycle.[1] Lizards, however, do not have a typical mammalian Cori Cycle. Instead, lizard metabolism is regulated by the primary goal of immediately replenishing muscle glycogen stores for subsequent activity.[11] This is because muscle glycogen depletion is considered a limiting factor in the intensity or duration of physical activity in reptiles.[15] To quickly refuel glycogen stores, lizards are able to perform gluconeogenesis in their muscles.

The first experiments to identify muscle gluconeogenesis in lizards were carried out by Gleeson.[13,15] Gleeson showed that red muscle is capable of glycogen synthesis from lactate *in vitro*, that the primary fate of lactate following exercise is gluconeogenesis, and that the primary substrate for glycogen resynthesis in muscle is lactate rather than glucose.[15] His series of experiments indicate that lizards use lactate in the muscle as a gluconeogenic substrate to directly replenish glycogen stores in the muscle. Unlike mammals, the liver plays no significant role in recovery metabolism. This unusual pathway of muscle gluconeogenesis has been shown in lizards, frogs and salamanders.[11]

As described earlier, gluconeogenesis in all species is an energetically expensive process and consumes ATP. Presumably, aerobic production of ATP by fatty acid metabolism fuels gluconeogenesis in lizards, as it does in mammals.[1] By using lactate as a substrate for muscle gluconeogenesis, lizards refuel their glycogen stores quickly rather than efficiently. This is because they need glycogen to fuel glycolysis and produce more ATP during the next burst of activity. Glycolytic ATP produced during lactate accumulation makes up 60-80% of total ATP produced during short periods of vigorous activity in lizards. In similar-sized mammals, only 5-

20% of ATP used during lactate accumulation comes from glycolysis.[11] Glycolysis during anaerobic conditions is therefore a much more important energy source for lizards than it is for mammals.

In mammals, glucose uptake is the limiting factor in glycolysis, and glucose uptake occurs through independent pathways stimulated by exercise and/or insulin. Conversely, glucose uptake is inhibited by exercise in lizards.[16] How can glucose uptake be inhibited under conditions that depend more heavily on glycolysis? Many studies using isotopic labeling have shown that lactate, rather than glucose, is the primary source of glycogen synthesis in lizards.[13,15,16] Since lactate produced during anaerobic conditions is undergoing gluconeogenesis to quickly refuel glycogen stores, glucose uptake into muscles is inhibited. Gluconeogenesis and glycolysis become a cyclic process. The substrates and products of each pathway fuel each other in a way that is similar to the Cori cycle, but the processes are isolated in muscle tissue.

If exercise inhibits glucose uptake in lizards, what effect does insulin have? In frogs, insulin increases lactate oxidation and oxygen consumption in muscle.[17] In rats, insulin inhibits lactate uptake and incorporation into muscle by 25 to 30% when glucose is present.[18] In mice, insulin stimulates glucose incorporation into muscle and has no effect on lactate metabolism.[19] Based on mammalian metabolism, insulin would stimulate glucose uptake. However, exercise has shown to have very different effects on glucose uptake in reptiles compared to mammals. It is impossible to predict what effect insulin will have without performing experiments. Experiments should be done that focus on the effects of insulin on glucose metabolism in exercised versus sedentary lizards. These results may help to define the pathways that insulin and exercise separately stimulate to cause glucose uptake in mammals.

An appropriate species of lizard to study glucose metabolism is the brown anole, *Anolis sagrei* (Figure 3). The brown anole and its congener, the green anole, *Anolis carolinensis*, have been well-studied models for exercise physiology. Brown anoles are a native species to Cuba and both genders display territorial behavior.[20] Brown anoles are invasive in Florida, the Bahamas, Jamaica and Belize. Wild-caught brown anoles from Florida are therefore easy to obtain for experimentation. Brown anoles exhibit the "sit and wait" predator strategy described above; they consume large prey in relation to their body size, and, after encountering prey, the lizards retreat quickly to avoid competitors.[20] This process of catching and digesting food requires large amounts of energy, which are replaced by muscle gluconeogenesis. As in the desert iguana, anole metabolism favors lactate over glucose as the primary substrate for glycogen synthesis.[21] Recovery rates for anoles average about 2.9 hours for a 5g lizard.[11] These factors combined make the brown anole a fitting model for studying glucose metabolism after vigorous exercise.

Figure 3. *Anolis sagrei*. Brown anole in its natural habitat.[35]

Anoles are also an appropriate model to treat with insulin injections. The hormone insulin was identified in the pancreas of the green anole in 1981. Pancreatic beta cells are second in abundance to alpha cells.[22] Alpha cells produce the hormone glucagon, which has reciprocal effects on regulation compared to insulin.[1] The lower quantity of beta cells supports experimental evidence that lizards are relatively insensitive to glucose.[22] While anoles may naturally produce less insulin, the role of insulin in lizard glucose metabolism is still undefined. Insulin receptors have so far been identified on the brain and liver of anoles. Receptors isolated in these two tissues show structural and mass differences similar to those seen in rat insulin receptors. Insulin receptors in the liver and brain are therefore highly conserved between reptilian and mammalian species.[23] This evidence suggests that anoles may similarly have insulin receptors within their muscle tissue.

Brown anoles are also interesting to study because a GLUT4 isoform is yet to be identified in a reptilian species. Since the insulin receptor has been conserved between reptiles and mammals, and GLUT4 is highly conserved among mammals[4], the GLUT4 transporter protein has potential for being conserved between mammals and reptiles as well. Quantifying GLUT4 receptors following treatments of insulin and exercise may help identify how these two treatments independently stimulate glucose transport.

Differentiating between the pathways of insulin-stimulated and contraction-stimulated glucose transport is important for medical applications. Glucose homeostasis is critical in patients with both type I and type II diabetes mellitus. Type I diabetes is an autoimmune disorder where the body fights off its own pancreatic beta cells and no longer produces insulin. Type II diabetes is the result of decreased insulin sensitivity. Both forms of diabetes use exogenous insulin as treatment to maintain glucose homeostasis. Regulating glucose transport through

exercise would also be a valuable therapeutic strategy. If doctors and scientists fully understood the mechanisms for exercise-stimulated glucose transport, they could prescribe treatment plans that do not depend on insulin injections. The cause of type I diabetes is still unknown and type II diabetes is becoming an epidemic.[7] Diabetes mellitus, therefore, is in need of both immediate and intensive scientific research.

MATERIALS AND METHODS

Study species. Adult male *Anolis sagrei* were collected in early January, 2009 from Glades Herps Inc. in central Florida. The anoles were transported by air to Colby College in Waterville, Maine, where all experimental procedures were performed in late January, 2009. Animals were housed individually in 12 qt Sterilite® plastic tubs. The tubs were used previously by MaryClaire McGovern[24] and had holes bored in the sides to allow sufficient airflow. Each tub was lined with Astroturf and contained a stick for perching and a water dish. The tubs were sprayed daily with distilled water to keep humidity levels high; daily temperatures ranged from 20-28ºC. The habitats were on light-dark cycles of 12-12h with full-spectrum lights. Anoles were fed twice weekly on a diet of two to four crickets per feeding and water was provided *ad libitum*. Prior to treatment, anoles acclimated for a minimum of two weeks; anoles were in captivity for up to four weeks.

Experimental design. The study species were assigned randomly into six groups of six anoles each; each group had the same mean mass. Lizards in Group A received no treatment and were not handled until dissection. Lizards in Group B were given placebo injections of saline on the day of experiment. Lizards in Group C were given injections of insulin. These individuals were never exercised and were used to measure the levels of metabolites following an injection of insulin. Lizards in Group D did not receive any injections and were exercised until they could be handled without restraint. Lizards in Groups E and F were also exercised to the same point; following exercise they were immediately administered the same injections as groups B and C, respectively. Specific parameters for the injected materials are defined below. Anoles were fasted 24 hours before receiving treatment.

Injections. Anoles in groups C and F received 15 IU/kg body mass of chicken insulin (courtesy of Dr. Jeffrey Bemis, Litron Laboratories, Rochester, NY). Doses of 15 IU/kg body mass were administered to stimulate a maximal response.[34] Chicken insulin was used because it is two to three times more reactive than porcine insulin.[23] Lizards and birds may also share a more recent common ancestor than lizards and mammals. Anoles in groups B and E received equivalent volumes of saline. Insulin and saline were injected into the base of the tail as demonstrated by Dr. Slack of the New England Animal Hospital in Waterville, Maine.

Figure 4. Exercise track. Brown anoles were exercised by running down the track.

Exhaustive exercise. Anoles were exercised on a track constructed previously by McGovern[26] of tempered-hardboard material, approximately 4.88m x 0.1m x 0.5m. Anoles were encouraged to run by prodding their tail and hind limbs with a stick, or waving a hand behind

them to simulate a predator approaching. Exhaustion was defined as the point where the anole could no longer be encouraged to move and could be picked up without resistance.

Dissection. Following appropriate treatment, each anole was immobilized in a 50-mL conical tube for 5 min. Anoles were then anesthetized in a 250-mL glass bottle on dry ice for 2 min before decapitation with a small razor blade. Timing of sacrifice following treatment mimicked a similar metabolic study done previously in rats.[25] Blood was taken from the severed neck using heparinized microhematocrit tubes as described previously[25,26] and analyzed immediately for glucose and lactate levels. The liver, quadriceps and illiofibularis muscles were removed using cooled dissecting scissors and forceps and immediately frozen in liquid N_2 for later analysis of glycogen and GLUT4 concentrations. Tissues were stored at -80ºC.

Glucose/lactate assay. Blood samples collected in capillary tubes were plunged into microcentrifuge tubes and spun at 3950 rpm for 30 s. Blood was analyzed for concentrations of glucose and lactate with a YSI 2300 STAT Plus glucose and lactate analyzer as described previously.[27] This instrument uses an electrode to measure the concentrations of glucose or lactate. A sample is injected into a buffer-filled chamber and diffuses through a polycarbonate membrane on a probe. When it contacts an immobilized oxidase enzyme, it is oxidized to produce hydrogen peroxide in the reaction: β-D-glucose + O_2 → glucono-δ-lactone + H_2O_2. Hydrogen peroxide is then oxidized at a platinum anode to produce electrons. When the probe reaches a steady state dynamic equilibrium of H_2O_2 production and oxidation, the electrons produced are proportional to the original concentration of glucose or lactate.

Glycogen assay. Muscle and liver tissue were measured for glycogen concentrations by enzymatic hydrolysis and chemical oxidation of glucose. This procedure is a modified version of previously described methods.[28, 29] Tissue samples were weighed individually on a Sartorius

analytical balance and then homogenized in 10 times tissue volume of 0.6 M cold perchloric acid on ice using a glass-glass homogenizer. 100 μL of the crude homogenate was removed and treated with amyloglucosidase (Sigma, catalog # A1602) to digest glycogen to glucose to measure total glucose. Supernatant from the remaining homogenate was neutralized to measure free glucose. Glucose concentrations were assayed using a glucose diagnostic kit (Sigma, catalog #GAGO-20). Samples were reacted with glucose oxidase to form gluconic acid and hydrogen peroxide. Hydrogen peroxide then reacted with o-dianisidine and peroxidase to produce a chromophore. The chromophore was reacted with H_2SO_4 to produce a more stable chromophore. The intensity of this pink chromophore, measured at 540nm with a Hitachi U-3100 spectrophotometer, is proportional to the original glucose concentration. Each sample was run in duplicate when possible. The mass-specific glycogen concentration of each sample was determined as the difference between total glucose and free glucose. Glycogen concentrations were converted into glycosyl units (μmol). Statistical analyses were conducted to compare treatment groups using Mann-Whitney U-tests on Vassarstats.

GLUT4 assay. Muscle tissue was analyzed for concentration of GLUT4 following a modified version of previously described methods.[30, 31] Muscle tissues were homogenized in 29 times volume of 10 mM HEPES, 1 mM EDTA, 250 mM sucrose, pH 7.4 on ice using a glass-glass homogenizer. An aliquot of the homogenate containing 50 μg of total protein was solubilized in Laemmli sample buffer. Samples were subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. GLUT4 was detected with rabbit polyclonal antibodies directed at the COOH-terminus of GLUT4 (F349; courtesy of Dr. Mike Mueckler, Washington University, St. Louis, MO) followed by HRP-conjugated anti-rabbit IgG (BioRad #170-6463). Quantification was assessed by relative color development.

RESULTS

A summary of all results is shown in Table 1.

Anole Mass, i	Anole Mass, f	%Δ mass	Tissue Mass	[Blood Glucose]	[Blood Lactate]	[Muscle Glycogen]	[Liver Glycogen]
No injection, sedentary							
3.26	3.35	2.7	0.0968	3.56	5.05	0.07	59.82
3.99	3.76	-6.1	0.0961	6.19	1.64	6.02	81.07
3.96	4.18	5.3	0.1245	4.29	1.34	1.86	48.50
2.15	2.4	10.4	0.0356	6.12	7.58	2.96	48.39
5.8	6.01	3.5	0.0631	1.93	0.42	4.08	27.59
3.49	3.46	-0.9	0.0654	7.53	4.57	6.44	93.42
Saline injection, sedentary							
3.18	3.38	5.9	0.0738	3.38	6.94	5.63	136.76
2.27	2.51	9.6	0.0519	2.51	7.09	1.57	119.91
5.57	6.38	12.7	0.1008	6.20	4.43	4.74	51.12
4.03	4.89	17.6	0.0903	7.44	8.16	5.14	28.84
3.94	4.35	9.4	0.0838	7.06	6.90	2.21	51.37
3.5	3.83	8.6	0.0410	3.83	6.44	1.42	93.40
Insulin injection, sedentary							
2.31	2.87	19.5	0.0668	2.87	8.01	2.61	36.66
5.05	4.83	-4.6	0.0715	6.47	6.08	4.03	41.71
3.87	3.31	-16.9	0.1491	6.96	5.71	2.50	14.48
3.17	3.46	8.4	0.0669	3.46	8.51	4.38	97.11
3.57	4.37	18.3	0.1067	4.37	7.44	2.70	94.00
4.05	4.3	5.8	0.1002	4.91	6.16	1.56	27.37
No injection, exercised							
4.08	4.49	9.1	0.0965	6.19	9.16	2.25	108.78
3.79	3.76	-0.8	0.0924	6.08	7.91	3.61	48.89
3.62	3.46	-4.6	0.0904	4.27	8.21	3.14	59.10
3.09	3.75	17.6	0.0889	4.43	7.97	3.31	74.09
4.87	4.84	-0.6	0.0850	5.43	8.82	6.86	12.35
2.33	2.58	9.7	0.0658	4.34	8.41	0.68	45.29
Saline injection, exercised							
3.78	3.78	0.0	0.1056	4.45	8.86	2.61	22.54
2.36	2.63	10.3	0.0534	3.46	7.78	2.88	42.82
3.08	3.13	1.6	0.0869	4.16	9.39	1.74	48.14
3.64	3.96	8.1	0.1297	2.74	6.22	1.88	86.10
4.41	3.94	-11.9	0.0724	4.99	9.78	2.75	23.33
4.59	5.66	18.9	0.1104	5.24	9.21	0.91	17.19
Insulin injection, exercised							
4.48	4.69	4.5	0.1084	5.00	11.50	2.27	88.98
4.18	3.85	-8.6	0.0782	6.70	12.50	1.11	66.28
3.66	3.57	-2.5	0.0950	5.74	8.00	2.06	34.82
2.74	2.8	2.1	0.0710	3.52	9.78	1.44	91.27
4.5	4.99	9.8	0.1344	5.43	9.08	7.00	52.64
2.55	2.86	10.8	0.0483	3.32	8.52	2.38	17.46

Table 1. Summary of data. Initial mass of *A. sagrei* prior to housing in laboratory (Anole Mass, i), final mass of anole after acclimating and before treatment (Anole Mass, f), and mass of combined quadriceps and illiofibrularis muscle or liver tissue (Tissue Mass) given in grams. Percent change in mass of anole (%Δmass) calculated as "Anole Mass, f" – "Anole Mass, i" ÷ "Anole mass, f" * 100%. Levels of glucose and lactate in blood plasma ([Blood Glucose], [Blood Lactate], respectively) given in mmol/L blood. Levels of glycogen in quadriceps and illiofibrularis muscle, or liver tissue ([Muscle Glycogen], [Liver Glycogen], respectively) given in μmol/gram quadriceps and illiofibrularis muscle, or liver tissue. Saline and insulin injections administered at 15 IU/kg body mass.

Blood glucose. Mean blood glucose levels did not differ significantly among treatment groups (P>0.05, Figure 5). Average blood glucose among all anoles was 4.85 ± 0.25 mmol/L.

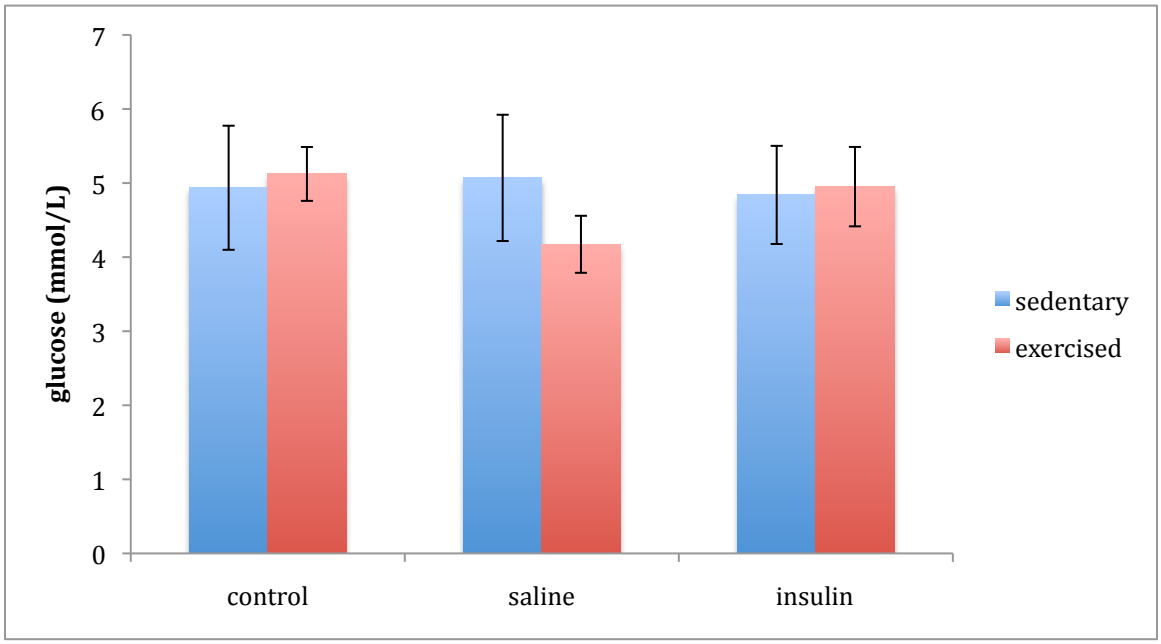

Figure 5. Blood glucose levels. Mean blood glucose levels in *A. sagrei* following treatment. Saline and insulin administered at 15 IU/kg body mass. No significant difference seen between any treatment groups (P>0.05). Error bars equal 1 SEM.

Blood lactate. Mean blood lactate levels were significantly lower in sedentary anoles compared to exercised anoles for all three treatments ($P<0.05$, Figure 6, Table 2). Mean blood lactate levels were higher for sedentary anoles that received an injection of saline or insulin than for the anoles in the control group, and for exercised anoles injected with insulin than those receiving saline or no injection, but the differences were not statistically significant.

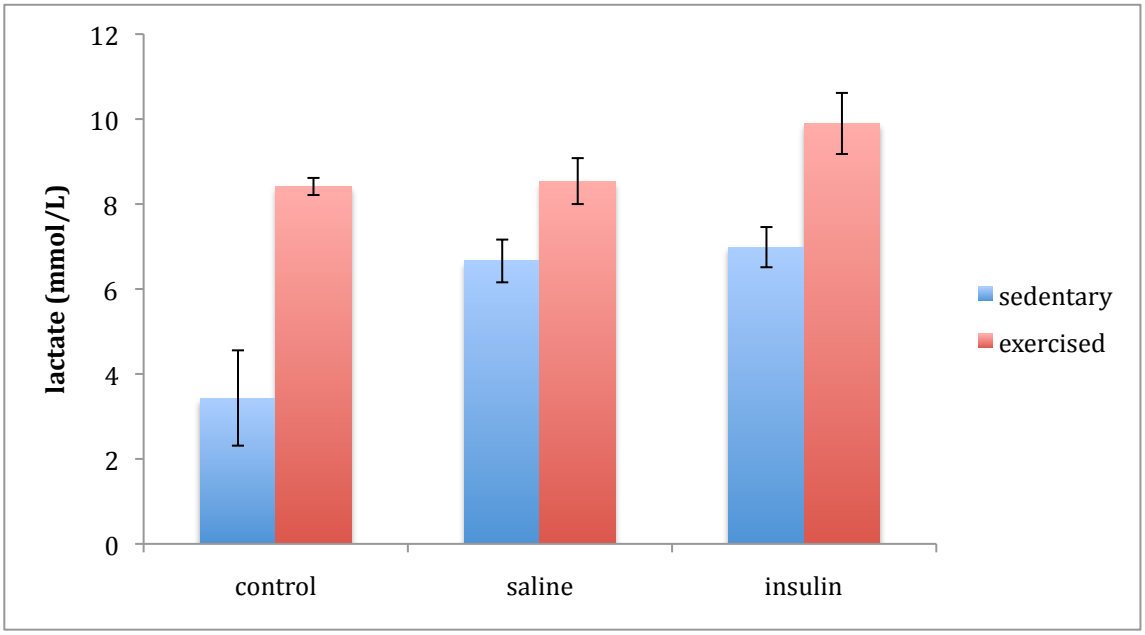

Figure 6. Blood lactate levels. Significant difference between *A. sagrei* in all treatment groups ($P<0.05$, Table 2) except saline/sedentary versus insulin/sedentary, control/exercised versus saline/exercised, and saline/exercised versus insulin/exercised ($P>0.05$). Error bars equal 1 SEM.

Group A	Group B	U_A	z	$P_{(1)}$	$P_{(2)}$
1	2	29	-1.68	0.0465	0.093
1	3	32	-2.16	0.0154	0.0308
1	4	36	-2.8	0.0026	0.0051
2	3	19	-0.08	0.4681	0.9362
2	5	30	-1.84	0.0329	0.0658
3	6	34	-2.48	0.0066	0.0131
4	5	23	-0.72	0.2358	0.4715
4	6	29	-1.68	0.0465	0.093
5	6	24.5	-0.96	0.1685	0.3371

Table 2. Statistical analysis of blood lactate levels following comparable treatments. U_A, z and P calculated by Mann-Whitney U Test of treatment groups listed in Group A versus B. 1= control/ sedentary; 2= saline/sedentary; 3= insulin/sedentary; 4= control/exercised; 5= saline/exercised; 6= insulin/exercised. Saline and insulin injections administered at 15 IU/kg body mass.

Muscle glycogen. Exercised anoles in the control and saline groups exhibited lower muscle glycogen levels than sedentary anoles in those treatment groups, but the differences were not significant (P>0.05). Muscle glycogen levels in exercised and sedentary anoles receiving insulin treatment did not differ significantly, and were, in fact, slightly higher in the exercised group (Figure 7, Table 1).

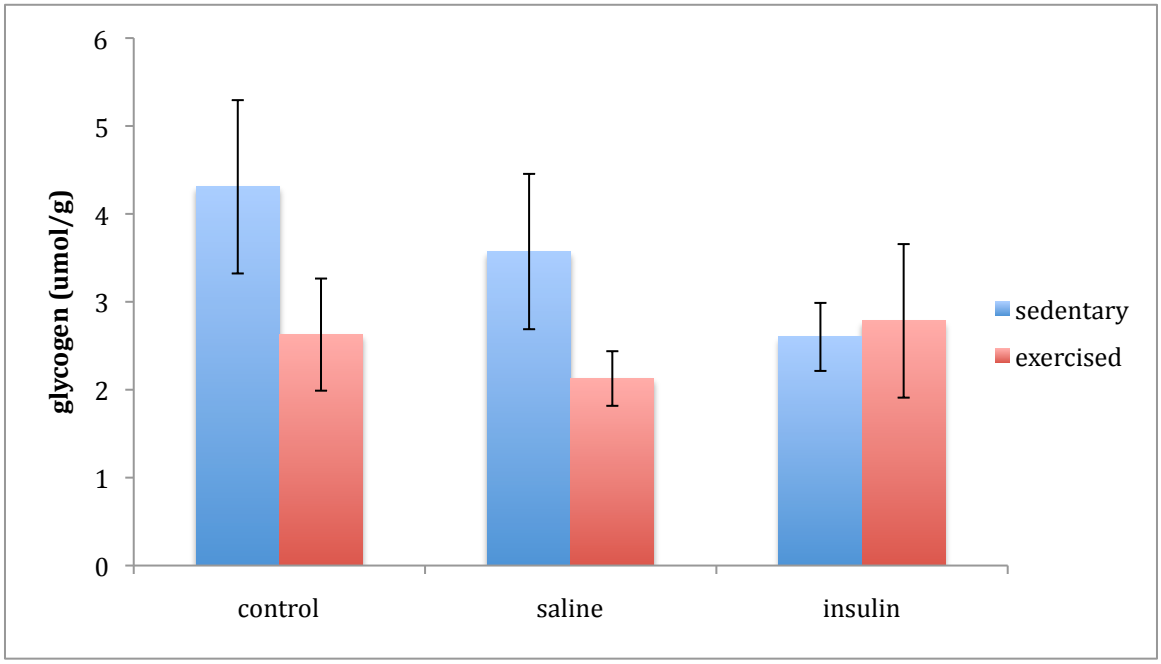

Figure 7. Muscle glycogen levels. Muscles assayed include quadriceps and illiofibularis from the hind limbs of *A. sagrei*. Saline and insulin injections administered at 15 IU/kg body mass. Significant difference between mean glycogen for control/sedentary group versus saline/exercised group (P=0.0329). Error bars equal 1 SEM.

Liver glycogen. Sedentary anoles injected with saline had the highest liver glycogen levels, followed closely by sedentary lizards in the control and insulin-injected groups. Exercised anoles in all three treatment groups had slightly lower mean liver glycogen levels than sedentary anoles, but the difference was only significant for anoles injected with saline (P = 0.046).

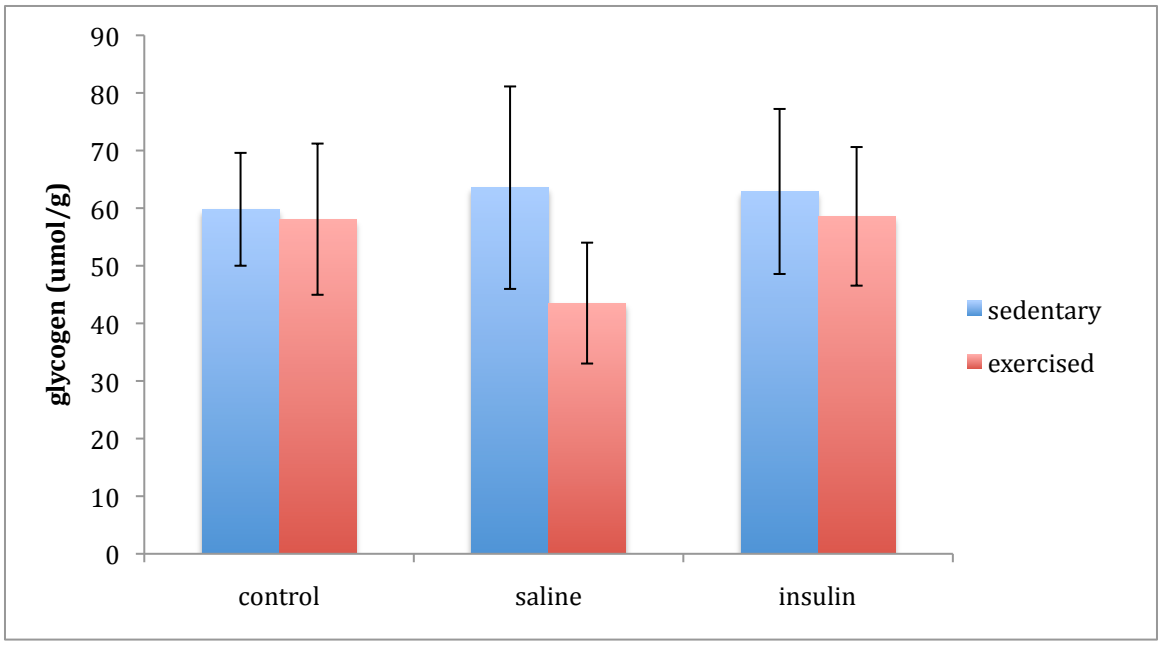

Figure 8. Liver glycogen levels. Saline and insulin injections administered at 15 IU/kg body mass. Significant difference between mean glycogen for control/sedentary versus saline/exercised group (P=0.0465) and between saline/sedentary versus saline/exercised group (P=0.0228). Error bars equal 1 SEM.

Muscle GLUT4. Detection by Western blotting produced no evidence of GLUT4. The primary anti-GLUT4 antibody was not detected by the secondary antibody, as the gels did not show any evidence of color development. The detection method was confirmed using 10 µg rabbit IgG, which showed a purple tone easily seen by the eye.

DISCUSSION

The purpose of this experiment is to determine how exogenous insulin and exercise individually and additively affect metabolism in the brown anole. The relative concentrations of blood glucose and lactate, muscle glycogen and GLUT4, and liver glycogen were compared following treatments. The ultimate goal is to learn more about how exercise stimulates GLUT4 through a different mechanism than insulin in mammals.

Glucose and lactate metabolism in the brown anole are greatly influenced by exercise. Muscle contraction requires anaerobic support, which produces lactate that undergoes gluconeogenesis within the muscle tissue to rebuild glycogen stores. Thus, the Cori Cycle is isolated in skeletal muscle tissue of the brown anole. The liver does not seem to play a role in recovery metabolism as there were no significant differences in liver glycogen levels after any of the treatments. The major changes in concentrations of metabolites as a consequence of exercise were primarily seen in skeletal muscle, as projected.

Data on control lizards served as a baseline for comparing lizards treated with an injected of saline or insulin. Average blood glucose levels were 88.9 mg/100 mL for the anoles in the control group. This concentration falls in the range of normal blood glucose levels in humans, between 60 and 90 mg/100mL.[1] Brown anoles therefore have comparable blood glucose levels as humans. The mechanisms by which brown anoles and humans maintain blood glucose homeostasis, however, are quite different. Following exercise, blood glucose levels fall in mammals because muscle contraction has stimulated glucose uptake.[1] This study on reptiles showed little or no decrease in blood glucose levels following exercise. These results indicate that exercise does not stimulate glucose uptake in muscle tissue, contrary to what has been reported for mammalian metabolism. This suggests that brown anoles may indeed be an ideal

model to help determine the mechanism by which muscle contraction stimulates glucose uptake in mammals. For example, in a future experiment, brown anoles and a mammalian model could both be exercised, then tissue would be removed for enzymatic analysis. Various enzymes suspected to play a role in the contraction-stimulated pathway could be monitored for their activity. If an enzyme is equally active in both models, it is probably not involved in the pathway. If an enzyme is activated in the mammal but not the anole, this enzyme may be involved in the pathway. By comparing the activities of various enzymes, the pathway by which muscle contraction stimulates glucose uptake in mammals could be determined.

The effects of exercise on blood glucose levels in brown anoles is similar to that observed in the lizard *Dipsosaurus dorsalis*.[13] At rest, blood glucose levels are 8.50 ± 0.484 mmol/kg on average in this reptile. After 5 minutes of exercise, blood glucose levels did not change more than 8% at any point during recovery between 30 and 120 minutes. This study supports the results found about the brown anole in the current study, that exercise does not stimulate glucose uptake. This further suggests that reptilian metabolism may help facilitate understanding the mechanism for the contraction-stimulated glucose uptake pathway seen in mammals.

Other studies in *Dipsosaurus dorsalis* have reported additional information to support the hypothesis that exercise does not stimulate glucose uptake in reptiles. Exercise may in fact inhibit glucose uptake. In one study, where the rates at which glucose and lactate incorporate into muscle glycogen following exercise were monitored, lactate was incorporated into muscle glycogen at a rate 11 times higher than glucose.[15] This provides support that lactate is a preferred substrate for glycogen synthesis. Depletion of glycogen stores limits the ability for lizards to perform exercise over long period of time, so their primary goal during recovery metabolism is to rebuild glycogen stores. Lactate produced from anaerobic metabolism is incorporated into

glycogen by muscle gluconeogenesis. Glucose uptake that normally occurs in mammals due to exercise does not occur in reptiles. Reptiles prefer lactate to glucose, so they do not need to take in glucose to make glycogen. The lactate substrate is already present in the muscle tissue. Glucose uptake into reptilian skeletal muscle tissue is therefore inhibited by exercise.

Lactate production in the brown anole significantly increased following exercise. Control anoles receiving no injections had blood lactate levels following exercise that were twice as high as those of control sedentary anoles. These results are again consistent in *Dipsosaurus dorsalis*, which showed an 11-fold increase in lactate levels following exercise.[13] Increased blood lactate following exercise is consistent across all animals as a result of insufficient oxygen delivery during exercise to oxidize pyruvate produced from glycolysis. When anaerobically metabolized, pyruvate is converted to lactate, which can be found throughout the whole body. Reptiles and fish have been shown to accumulate lactate at higher rates than mammals and some amphibians.[16] In *Dipsosaurus dorsalis*, lactate levels remain elevated even when oxygen levels return to the level at rest.[10]

Lactate has a different fate between classes of animals. In mammals, lactate is either oxidized or used as a substrate for gluconeogenesis, although the latter process is energetically expensive. In reptiles, lactate is primarily converted to skeletal muscle glycogen by gluconeogenesis. In *Dipsosaurus dorsalis,* 50% of lactate produced during exercise is used to synthesize glucose and glycogen. Lactate removal and glycogen production occur at proportional rates and in the same approximate time period. Mammals, on the other hand, exhibit depleted glycogen levels long after accumulated lactate has been metabolized.[15] This evidence further suggests that lactate is the key substrate in recovery metabolism, and that increased glucose uptake is not critical in the brown anole.

Muscle glycogen levels in the brown anole did not differ significantly between sedentary and exercised anoles; however, a notable trend was seen in which glycogen levels decrease following exercise. These results have been shown in another prior study on the brown anole.[24] The same study also reported that muscle glycogen levels increase and begin to return to the resting concentration after a 20-minute recovery period. In *Dipsosaurus dorsalis*, glycogen levels decrease following exercise, begin increasing after 30 minutes of recovery, and then rise above resting levels and remain elevated for at least 120 minutes.[13] All three of these studies show the same changes in reptilian muscle glycogen due to exercise, and it is clear that exercise first causes glycogen to be broken down to glucose monomers that undergo glycolysis to produce ATP and fuel muscle contractions. After an adequate recovery period, lactate undergoes gluconeogenesis to rebuild glycogen stores.

Further evidence to confirm that gluconeogenesis occurs in reptilian muscle tissue is suggested by liver glycogen levels in the brown anole. There were no significant differences in mean liver glycogen levels between sedentary and exercised anoles. This suggests that the liver plays no role in recovery metabolism. *In vitro* studies on metabolism in frogs, salamanders, and other lizards also suggest that the liver plays no significant role.[11] In *Dipsosaurus dorsalis*, isotope labeled lactate showed negligible incorporation into liver glycogen during the first 90 minutes of recovery.[13] Again, this result confirms that gluconeogenesis does not occur in the liver. Isotope labeled glucose was detected in liver glycogen but no amount was detected in blood lactate. [13] These results support the idea that blood glucose is not being transported into the muscle tissue during exercise. If labeled glucose was being transported into the muscle, it would have undergone anaerobic metabolism and appeared as blood lactate. Glucose is instead being transported into the liver for glycogen synthesis, rather than into the muscle. All changes in

metabolite concentrations discussed thus far indicate that glucose uptake into muscle is not stimulated by exercise in the brown anole.

It was originally hypothesized that the brown anole has a GLUT4 transporter protein similar to mammals. The transporter protein is stimulated by exercise to transport glucose into muscle cells in mammals. However, because all of the measured metabolites in the brown anole suggest that glucose transport is not stimulated from exercise, the brown anole may not have an isoform of the GLUT4 protein. Western blot analysis detected no levels of GLUT4. This may be because the primary antibody used was raised specifically against chicken GLUT4. Extensive studies have shown, however, that GLUT4 is highly conserved among species.[4]

More likely, brown anoles do not have a GLUT4 isoform in their muscle tissue. Even if the primary antibody was unreactive between species, other metabolites would have indicated if GLUT4 was indeed present. Following exercise, there would have been a decrease in blood glucose, an increase in glucose transported into muscle cells, and then transported glucose would have been incorporated into newly synthesized glycogen. In the brown anole, blood glucose levels did not change due to exercise. Isotope labeling studies down in the *Dipsosaurus dorsalis* further indicate that blood glucose is not incorporated into muscle glycogen following exercise.[13] Thus, GLUT4 proteins are apparently not present or are unaffected by exercise.

Another indicator of GLUT4 proteins is a response to insulin. GLUT4 is insulin sensitive, so anoles being injected with insulin were expected to show increased translocation of GLUT4 and then increased movement of glucose from the blood to the muscle tissue. However, brown anoles appeared to have no metabolic response to insulin. Five minutes post injection, blood glucose, blood lactate, liver glycogen and muscle glycogen levels were not significantly different than anoles that received no insulin treatment. This indicates that insulin did not stimulate

GLUT4 translocation, and further suggests that GLUT4 may not even be present in the brown anole. There are many other reasons why insulin may have caused no metabolic response, which further experiments can confirm.

Results reported here may have been influenced by undetected experimental errors, such as using potentially degraded insulin because it was originally purified in 1987. It was theoretically stored at -20°C; but, Litron Laboratories no longer sells insulin commercially so they could not guarantee the insulin is still reactive. Other studies, however, have used insulin from Litron Laboratories in the past few years and have had a successful response.[32,33]

Too little insulin may have been administered, so that no response from treated lizards was stimulated. The concentration of 15 IU / kg body mass has shown a maximal response in toads.[34] Reptiles may require a higher dose to stimulate the same response. Injecting the anoles with various concentrations of insulin and monitoring its effect on blood glucose levels would determine the ideal dose to inject into anoles. One consideration in doing this type of experiment is to avoid causing the anoles to enter a hypoglycemic state. Hypoglycemia is the result of abnormally low blood glucose levels. This can be caused by an overdose of insulin, defects in the gluconeogenesis pathway, or exercise (particularly in diabetics). Hypoglycemia results in discomfort, and mental confusion, and can lead to permanent brain damage or death in mammals. Glucose is the brain's sole source of energy; drastically low levels of glucose circulating in the bloodstream means the brain is no longer getting enough glucose to function properly. Other tissues are also affected by hypoglycemia. When glucose is no longer available to produce ATP, fat and protein stores are metabolized. Unintentionally activating lipid and amino acid metabolism would negatively affect an experiment intended specifically to study

glucose metabolism. Hypoglycemia should therefore be avoided in future experiments aimed to determine the optimal dose of insulin needed to trigger a significant metabolic response.

Insulin used in this study may also have been unreactive because the hormone was purified from chicken pancreas. Chickens and lizards share a recent common ancestor, but chicken insulin may not be cross-reactive between the two species. Chicken insulin has, however, been shown to be two to three times more reactive than porcine insulin in the green anole.[23] Although this suggests that chicken insulin should also be reactive in the brown anole, the results of this experiment suggest otherwise. Chicken insulin used in this study and in the study cited for its relative potency were both from Litron laboratories; however, they were most likely purified at different times and one lot may have been more cross-reactive than another. Factors including purity, temperature, and pH, could have caused two lots of chicken insulin to have different properties.

The binding of chicken insulin to anole insulin receptors has shown to be pH, time, and temperature dependent. Maximal specific binding has been seen at 4°C, after 4-18 h, and at pH 7.8.[23] Physiological temperature and pH are essential for this study, but future experiments could compare the reactivity of insulin after various lengths of time. This study only allowed 5 minutes for insulin to react, which could have been substantially too short. Waiting longer between injection and decapitation may have allowed for a notable response to occur.

Most notably, reactivity between chicken insulin and anole insulin receptors has only been documented for insulin receptors in the brain and liver.[23] Reactivity of chicken insulin with insulin receptors on skeletal muscle tissue is yet to be studied. Furthermore, no study has yet to even confirm the presence of insulin receptors in skeletal muscle tissue in the brown anole. This issue would be the first hurdle to overcome in future experiments. Insulin receptors on skeletal

muscle tissue could be identified by immunoassay, reverse-transcriptase real-time PCR (RT-PCR), using radiolabeled isotopes of insulin, or another detection techniques. Confirming the presence of insulin receptors in skeletal muscle is absolutely critical to studying the effects of insulin on muscle metabolism. It is possible that brown anoles do not even have insulin receptors on their skeletal muscle. More likely, it is possible that brown anoles have insulin receptors but insulin does not stimulate glucose uptake. Insulin plays many other critical roles, including regulating lipid metabolism, amino acid metabolism, enzyme activity, DNA replication, and protein synthesis. If brown anoles indeed do have insulin receptors, further experiments should compare various sources of reptilian and mammalian insulin to determine which type provokes the most significant response. Insulin purified from brown anole pancreases would be ideal for this study.

If insulin does bind to insulin receptors on muscle tissue in the brown anole, future studies should look deeper into the presence and activity of a GLUT4 isoform. Results discussed so far suggest that GLUT4 is not present in the brown anole. If GLUT4 is present, it is almost certainly not activated by exercise, but could still be activated by insulin, especially if insulin receptors are found on the muscle tissue. Studies should first look to identify the presence of a GLUT4 isoform. Western blots, RT-PCR, and other detection techniques could be used. If a GLUT4 isoform is identified, it would be interesting to see the combined effects of exercise and insulin on GLUT4 activity in the brown anole. Exercise has been shown to inhibit typical GLUT4 activity, because muscle gluconeogenesis inhibits glucose uptake. Perhaps the GLUT4 isoform evolved in the brown anole differently than in mammals. Because exercise inhibits glucose uptake into muscle tissue, GLUT4 in the brown anole may not activate the same enzymes that are activated in mammals that cause contractions to stimulate glucose uptake.

Enzymes activated by GLUT4 following exercise could be compared between the brown anole and mammals. Enzymes activated in mammals but not in anoles may be involved in the pathway by which contractions stimulate GLUT4 in mammals.

CONCLUSION

Anolis sagrei exhibit similar metabolic responses to exercise as the well-studied lizard, *Dipsosaurus dorsalis.* Following exercise, muscle glycogen stores are broken down to make ATP and fuel muscle contractions. As a result of anaerobic metabolism, lactate is produced at high rates. Lactate then undergoes gluconeogenesis in the muscle tissue to quickly rebuild glycogen stores. The liver plays no significant role in recovery metabolism. Future studies in these reptilian species have great potential to help discover the mechanism of the contraction-stimulated glucose uptake pathway in mammals. If a GLUT4 isoform is present in reptiles, it probably does not facilitate diffusion of glucose into muscle cells following exercise as it does in mammals. Its effects may be inhibited due to muscle gluconeogenesis. Comparing muscle enzyme activity following exercise between mammals and reptiles would show which enzymes might be activated in mammals to cause increased glucose uptake. If a GLUT4 isoform is not present in reptiles, similar studies on enzyme activities can also be done. Enzymes activated by exercise in mammals, but not activated by exercise in reptiles, may be involved in the contraction-stimulated glucose uptake pathway. These potential studies stem from a critical finding: exercise does not stimulate glucose uptake in reptiles as it does in mammals. A reptile, therefore, may be an ideal model for discovering the mechanism by which contractions stimulate glucose uptake in mammals. This discovery could lead to alternative treatment methods in both type I and type II diabetes. Replacing insulin injections with regimented exercise routines would be more convenient, less expensive, and undoubtedly safer for the body.

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