

THE EFFECT AND DEPENDENCY OF L-THYROXINE TREATMENT ON THE  
BASAL METABOLIC RATE OF POST-METAMORPHIC *XENOPUS LAEVIS*.

A Report of a Senior Study

by

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Editor



## ABSTRACT

One function of thyroid hormones is stimulating both the anabolic and catabolic reactions that make up an organism's metabolism. However, the thyroid may not function properly resulting in hypothyroidism. One side effect of hypothyroidism is a declination in metabolic rate. This study investigated if the thyroid hormone l-thyroxine ( $T_4$ ) could significantly increase the metabolic rate of *Xenopus laevis* frogs. It was hypothesized that the thyroxine treated *Xenopus* would demonstrate an increased metabolic rate and decreases in mass. Further, it was hypothesized that after treatment was halted, the experimental group was expected to demonstrate a decrease in metabolic rate and an increase in mass. Twenty-two *Xenopus* tadpoles were treated with 1mg of l-thyroxine per liter of water for 21 days, and treatment was withdrawn for 21 days. Similarly, twenty-one control tadpoles were treated with 1%NaOH for 21 days. Throughout the 42-day period, the whole body and mass specific metabolic rate was measured using gas exchange respirometry. There was significant increase ( $p=0.04$ ) in whole body metabolic rate of  $T_4$  treated animals, but the increase was not restricted to the treatment period ( $p=0.12$ ). There was no significant increase in mass specific metabolic rate. Also, there was no significant difference in mass between the final control and the final thyroxine treated ( $p=0.16$ ). These results suggest that l-thyroxine may play a significant role in frog metabolism even though there was not a significant difference mass or mass-specific metabolic rate.

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## CHAPTER I

### INTRODUCTION

#### Metabolism and Metabolic Rate

Metabolism is defined as the sum of all chemical processes that occur within an organism (Randall, Burggren, & French, 2002), and there are two broad categories that are used to describe metabolic reactions: anabolism and catabolism (Weston, 1992). Anabolism is when small materials combine to synthesize a large structural or functional unit through the input of energy. Conversely, catabolism takes large molecules and breaks them down while releasing energy. These catabolic products are then used for the anabolic pathways. Two other metabolic terms described by Weston (1992) are amphibolic and anaplerotic. Amphibolic is a pathway where anabolism and catabolism are utilized. Anaplerotic is a process where intermediates are replenished as they are being used by metabolic pathways. Energy produced from metabolic pathways can be stored, used for work, or lost as heat. Therefore, an organism's metabolic rate can be represented as the amount of heat produced from the conversion chemical energy. The metabolic rate of an organism is dependent on body temperature, environmental temperature, body mass, reproduction, activity, time of day, season, age, sex, shape, stress, and food (Randall, Burggren, & French, 2002).

Metabolic rates can be expressed as basal metabolic rate (BMR), standard metabolic rate (SMR), or field metabolic rate (FMR) (Randall et al., 2002). BMR is when the organism is under little stress and there are no digestive processes occurring. For organisms that are not

birds or mammals, this rate also requires a controlled body temperature in which the organism does not have to further regulate its body temperature. However, standard metabolic rate (SMR) is the metabolic rate under the same conditions as BMR at a given body temperature. The two metabolic rates give are useful to compare rates among and between organisms. Though, a third form of metabolic rate is needed in order to represent natural conditions. The field metabolic rate (FMR) is the average metabolic rate of an organism in natural conditions. This rate accounts for both resting rates and rates during maximum physical activity, but is often difficult to attain accurate results.

#### Methods for Measuring Metabolic Rate

Three techniques used to measure metabolic rate are calorimetry, radiolabeled isotopes, and respirometry. For calorimetry, there are two forms of measurements: direct and indirect. Direct calorimetry determines the metabolic rate by measuring the amount of heat emitted from an organism (Randall et al., 2002). This method is based on Hess's law that states the breaking down of fuel into a particular product releases the same amount of energy independent of the chemical pathway used. To measure the amount of heat released by an organism, it is placed within a completely insulated chamber. The change in temperature inside the chamber is measured to determine the amount of energy lost from the organisms. This value is expressed in calories or kilocalories per hour. However, Randall et al. (2002) mention that this method does have limitations. Some organisms have a very low metabolic rate that leads to unreliable results. Also, some organisms may be too large to place within a chamber. Indirect calorimetry measures the metabolic rate by subtracting the amount of energy excreted from the amount ingested. Food and excretion materials are placed in a bomb calorimeter to determine the heats of combustion. This technique also has drawbacks. For instance, not all food ingested can be



digested by the organism, and this must be factored into the results. In addition, only a generic metabolic rate can be determined. Therefore, this technique is not useful in determining the basal, standard or resting metabolic rate

Another technique to measure metabolic rate is the use of radiolabeled isotopes. A water radioisotope mixture is injected into an organism, and the rate of radioactivity deterioration is measured over time (Randall et al., 2002). Another common isotope used in metabolic studies is carbon-14, which is used to replace the carbons in glucose (Weston, 1992). This technique is capable in determining the field metabolic rate because organisms can behave without restrictions (Randall et al., 2002).

The third type of metabolic measurement is respirometry (Randall et al., 2002). Instead of calculating the amount of heat produced, respirometry measures the amount of oxygen consumed or carbon dioxide produced. This method is based on the fact that oxidation is required to gain energy from food. Thus, oxygen consumption is directly related to heat production during aerobic oxidation. To determine oxygen intake or carbon dioxide production there are three types of systems employed: closed, open or a combination of the two. In closed-system respirometry, the organism is placed within a closed chamber filled with either air or water. Then the amount of oxygen depleted or the amount carbon dioxide added to the container is monitored over a set time period using an oxygen or carbon dioxide electrode. For an open system, the organism is placed in a medium that is capable of leaving the chamber. Respiratory exchange is calculated by measuring the gas concentrations or partial pressures as gas or water flows into or out of the chamber. These two types of respirometry can be combined in order to determine the gas exchange of separate body sections simultaneously. However, for respirometry to be accurate three assumptions must be met: (1) the chemical

reaction must be aerobic, (2) the energy released during the consumption of a given volume of oxygen is the same for all energy sources, and (3) the organism is not capable of storing large quantities of oxygen.

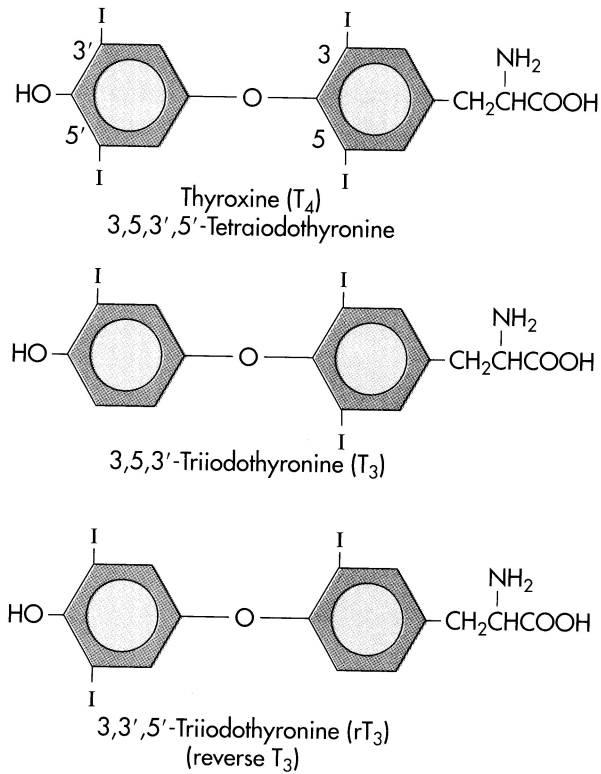
### Comparative Metabolic Rate

Metabolic rates vary greatly among the different classifications of organisms (Randall et al., 2002), with most differences being based on how heat is produced. Endotherms produce heat by means of metabolism, while ectotherms gain heat from the environment. Endotherms usually maintain a body temperature above the environment, which demands large amounts of energy input. Since metabolism is required to produce heat, endotherms have higher metabolic rates compared to ectotherms. Humans, birds, and other mammals are examples of endotherms. Ectotherms tend to have body temperatures close to the ambient temperatures, thus requiring less energy input. As a consequence, they have relatively low metabolic rates. Examples of ectotherms are invertebrates, fish, reptiles, and amphibians. Metabolic rate also varies among endotherms and ectotherms. The most significant factor is the size of the organisms. Among similar types of animals, smaller organisms have a higher metabolic rate for their mass.

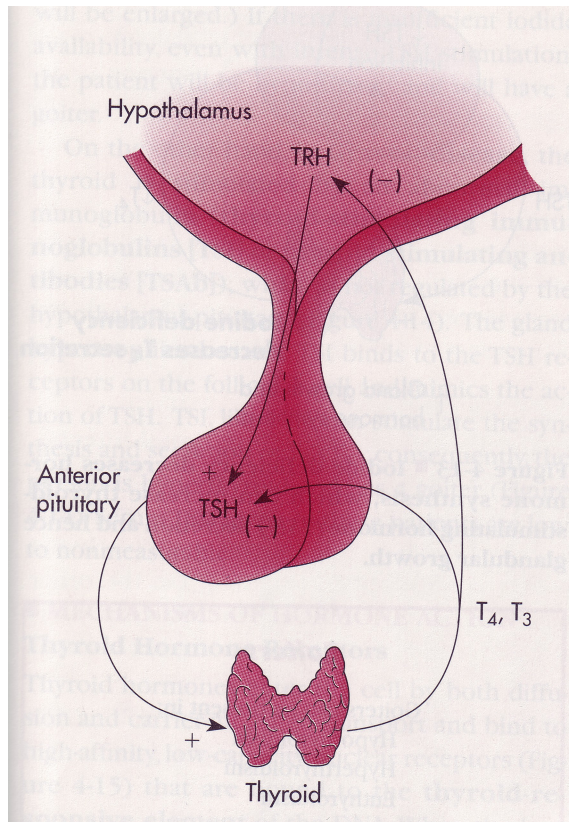
### Thyroid Gland and Thyroid Hormones

The endocrine system plays an important role in maintaining metabolism. The thyroid gland is responsible for producing the hormones needed for metabolic regulation (Porterfield, 1997). According to Porterfield (1997), the thyroid is made up of follicular cells, “C” cells and colloid material. Follicular cells function is to capture and concentrate iodide, a necessary component of thyroid hormones. The “C” cells are responsible for producing calcitonin. Thyroglobulin, which is a precursor to thyroid hormones, makes up the colloid material. There are three important thyroid hormones: Thyroxine ( $T_4$ ), triiodothyronine ( $T_3$ ), and reverse  $T_3$  as

shown in Figure 1. Secretion of thyroid hormones is regulated by the thyroid-stimulating hormone (TSH) made in the pituitary gland. Figure 2 is a pictorial showing the hormone pathway through the hypothalamus to the thyroid and back to the hypothalamus.



**Figure 1. Structure of the three thyroid hormones (Porterfield, 1996, 59).**



**Figure 2. The pathway of the main thyroid hormones (Porterfield, 1996, 65).**

Thyroid hormones cause many cellular-level changes that increase the metabolic rate of organisms by influencing virtually all metabolic pathways (Storey, 2004). The greatest influence is the hormones effect on the utilization of energy. However, the hormone effects are not immediate like insulin. Instead, they use direct action or altering other regulatory hormones to affect energy expenditure over the medium or long-term. Because thyroid hormones increase energy expenditure, they also increase heat production. Heat production is linked to increased consumption of oxygen. Thus, the metabolic rate increases as well. If there is excessive thyroid hormone present, the metabolic rate is affected. Furthermore, these hormones also stimulate both anabolic and catabolic pathways Thyroid hormones trigger both protein synthesis and degradation (Porterfield, 1997). Normal levels of thyroid hormone stimulate protein synthesis, by increasing cellular uptake of amino acids and incorporating the acids into proteins.

However, protein catabolism occurs when excess thyroid hormone is present (Storey, 2004). Thyroid hormones also affect both lipid synthesis and degradation (Porterfield, 1997). However, thyroid hormones have more influence on degradation because hormone-sensitive lipase function is intensified. With higher hormone levels, the amount of overall lipids decrease. Finally, thyroid hormones affect carbohydrate metabolism by playing a role in managing glucose levels. Overall, glucose levels remain normal, but excess hormones causes a sharper increase in glucose levels immediately after glucose intake. This could be the result of higher glucose absorption or decrease in insulin efficiency. In general, most of the cellular changes mentioned lead to an increase in energy expenditure and an elevated metabolic rate.

Different organisms have specialized metabolic functions according to their needs (Gorban, Dickhoff, Vigna, Clark, & Ralph, 1983). For humans, the thyroid hormones are responsible for regulating the basal metabolic rate (Porterfield, 1997). In other animals, the thyroid also has other metabolic functions other than those previously mentioned. Thyroxine is essential for amphibian metamorphosis (Gorban et al., 1983). If thyroxine is in excess, amphibians will morph too soon producing immature frogs. However, if no iodide is available and thus no thyroxine is formed, amphibians will not undergo metamorphoses. In other animals, including humans, thyroid hormones play a crucial role in growth and development. Infants with sporadic congenital hypothyroidism will not undergo normal brain development that can result in severe brain damage (Porterfield, 1997). Experiments have shown that thyroid hormone  $T_4$  have an affect on growth hormones (Gorban et al., 1983). The exact mechanism is unknown, but there are two leading theories. Either  $T_4$  makes tissues more sensitive to growth hormone, or  $T_4$  regulates the amount of growth hormone produced. Children with deficient circulatory thyroid hormone concentrations have delayed bone development, stunted growth,

and experience a delayed or absent puberty (Porterfield, 1997). However if thyroid hormone replacement is initiated early enough, these developmental dysfunctions can be reversed. Overall, the metabolic roles of thyroid hormones are regulation of oxygen consumption, synthesis and degradation of macromolecules, and growth and development of organisms.

### Metabolic Diseases of the Thyroid

In humans, there are several disorders associated with the thyroid glands and its production of thyroid hormones. Two major disorders are hyperthyroidism and hypothyroidism (Porterfield, 1997). Hyperthyroidism, also called Grave's disease, is characterized by the over production of thyroid hormones (Duncan, 1959). Symptoms of the disorder include palpitation, tachycardia, exophthalmoses, goiters, nervous restlessness, excessive sweating, diarrhea, amenorrhea, and emaciation. The basal metabolic rate of those with hyperthyroidism is usually higher than normal. The disease is usually more common in females and prevails in ages between 30 and 50. Stress and psychological trauma have been seen to cause the onset of the symptoms. Duncan (1959) discusses that the treatments for hyperthyroidism consist of removing part of the gland, iodine therapy, and the use anti-thyroid drugs.

Another disease associated with the thyroid gland is hypothyroidism (Kasper, 2005). Kasper describes hypothyroidism as a decrease in thyroid function and hormone production. The disorder can be subdivided into two types. First, subclinical hypothyroidism is when the concentration of thyroid hormones is normal, but there are high levels of thyroid stimulating hormone. On the other hand, the gland may not be able to generate enough hormones despite high TSH levels known as clinical hypothyroidism. Porterfield (1997) describes that hypothyroidism may be caused by the destruction of the gland, inhibition of hormone synthesis, hypothalamic or pituitary disorders, or resistance to thyroid hormone. Symptoms of

hypothyroidism include a lower basal metabolic rate, intolerance to the cold, dry skin, weight gain, constipation, goiter, fertility complications, and thin, brittle hair and nails. Symptoms are usually mild in subclinical while more severe in clinical hypothyroidism. Children who have hypothyroidism exhibit a delay in physical and mental growth and development. Again, women are more likely to develop hypothyroidism than men. Duncan (1959) explains that there is no cure, but treatment for hypothyroidism is thyroid hormone replacement therapy. The three most common hormones used in such therapy are (1) desiccated thyroid, (2) l-thyroxine, (3) l-triiodothyronine.

#### L-Thyroxine Treatment of Hypothyroidism

L-thyroxine is a common treatment for clinical hypothyroidism (Duncan, 1959). Due to the simplicity of this molecule, L-thyroxine has the ability to be dispensed orally in fixed quantities, and its materials can be identified chemically. This treatment is also capable of suppressing uptake of radioactive iodide, reduce non-toxic goiter size, and maintain normal thyroid hormone levels. Also, L-thyroxine increases the basal metabolic rate and decreases cholesterol levels. Those treated with thyroxine are capable of maintaining normal hormone levels throughout their lives while receiving treatment. Thus, those who suffer low thyroid hormone levels are dependent on hormone replacement.

Several studies have investigated the use L-thyroxine therapy treatment of subclinical hypothyroidism. Jaeschke, Guyatt, Gerstein, Patterson, Molloy, Cook, Harper, Griffith, and Carbotte (1996) investigated whether the health-related quality of life (HRQL) improves for middle age or older patients receiving L-thyroxine for subclinical hypothyroidism. Thirty-seven patients with symptoms consistent with hypothyroidism were given either L-thyroxine treatment or a placebo in a double blind study. The study measured hormone levels, disease-

specific and general HQRL, cognitive function, bone mineral density and lipid levels. In treated patients, TSH levels decreased while T<sub>4</sub> increased. Psychometric memory tests showed a significant improvement with treatment. All other measurement showed similar results between the experimental and control groups. There was a trend showing HQRL improvement; however, the trend was not statistically significant. In a similar study, Nystrom, Caidahl, Fager, Wikkelso, Lundberg, Lindsted (1988), investigated the effect of L-thyroxine treatment on 20 women with subclinical hypothyroidism using a double-blind study. Treated patients exhibited a significant increase of procollagen-III-peptide concentration. Procollagen-III-peptide increases correlate with increased free thyroxine, total thyroxine, and reverse triiodothyronine. After treatment was halted, four patients remained clinically euthyroid for up to 6 months. Using psychometric testing and self-judgment, it was found that approximately one in four women improved from therapy. Ozcan, Ckair, Yaman, Akgul, Erturk, Beyhan, Bilgi, and Erbil (2005) evaluated the effects thyroxine treatment had on biochemical indicators of cardiovascular disease risks. The study evaluated the plasma concentrations of total homocysteine (tHcy), high sensitive C-reaction protein (hsCRP), small low-density lipoprotein (sdLDL), L-arginine, and asymmetric dimethylarginine (ADMA), and their relation to the production of nitric oxide (NO<sub>x</sub>) in the plasma. Those treated were 84 women with subclinical hypothyroidism and 33 healthy women controls. Before treatment, the women with hypothyroidism had a significantly lower concentration of NO<sub>x</sub> and higher total cholesterol, LDL-C, hsCRP, ADMA, and L-arginine than the control. These conditions have been linked to increase risk of developing cardiac disease. After treatment ADMA and hsCRP concentration decreased, and sdLDL was also lowered. However, total cholesterol and LDL were elevated. It



was concluded that decreasing ADMA and hsCRP, increases NO<sub>x</sub> concentrations. Insufficient NO<sub>x</sub> is connected to cardiovascular dysfunction, and treatment is beneficial.

### L-thyroxine and Amphibian Studies

The aforementioned studies show that L-thyroxine treatment is effective at raising circulating thyroid hormone concentrations, but a question arises whether this treatment is effective at raising metabolic rate. Thus, it can be questioned whether L-thyroxine treatment would increase the basal metabolic rate, or if L-thyroxine has an effect independent of the metabolic rate. An example of such metabolic rate independence by T<sub>4</sub> was shown by Griswold, Fischer, and Cohen (1972) who looked at temperature dependency among the intracellular distribution of thyroxine in the liver of *Rana catesbeiana* tadpoles. Radiolabeled thyroxine was used to determine liver cell uptake of thyroxine and intracellular uptake into the liver cell nucleus. The results suggest that intracellular uptake into the nucleus is inhibited in cold temperatures; thus, thyroxine induced metamorphoses was also inhibited. Another study done by Chang (1957), looked at the role of thyroxine in color control mechanism in *Xenopus laevis*. Chang (1957) conducted several experiments looking at melanophore contraction to determine the mechanism on which thyroxine acts. In one experiment, *Xenopus* were treated either by placing them in varying concentrations of L-thyroxine Na solutions or injecting the solution within a body cavity. In both cases, the frogs were blanched, and injections achieved blanching more rapidly. Follow up experiments were done to determine whether thyroxine directly or indirectly affects the melanophores. The results suggest that thyroxine acts on melanophores indirectly through a neurohumor cholinergic mechanism. The study concluded that thyroxine may play an important role of amphibian endocrinology, but the affect of T<sub>4</sub> on whole-body metabolic rate is unknown. This study will look at the effectiveness and

dependency L-thyroxine has on the basal metabolic rate using the model species *Xenopus laevis*.

### Experimental Proposal

*Xenopus laevis* was chosen as the model organism for this study due to the fact that amphibians have a thyroid gland capable of producing thyroid hormones (Deuchar, 1975). Timing of thyroxine exposure is also important due to the tremendous effects that thyroid hormones have on amphibian development during metamorphoses (Duellman & Trueb, 1986); because of this, post-metamorphoses frogs will be examined. In this study, metabolic rate and metabolic dependency will be evaluated for L-thyroxine treated *Xenopus*. It is hypothesized that the thyroxine treated will demonstrate an increased metabolic rate and decreases in mass. After treatment is halted the experimental group is expected to demonstrate a decrease in metabolic rate and an increase in mass.

## CHAPTER II

### MATERIALS AND METHODS

#### Animal Husbandry

Forty-three *Xenopus laevis* tadpoles (stage 62-64) were obtained from Nasco (Fort Atkinson, WI). In order to prevent shock, the tadpoles were acclimated for approximately 15 minutes at room temperature water. The tadpoles were randomly divided into groups of five or six and placed into plastic containers filled with 3L of tap water dechlorinated with Kent Fresh/Marine Ammonia Detox (1ml /5 gallons). Overall 21 tadpoles were designated as control and 22 experimental. Throughout the experiment, a 100% water change was done every Monday and Thursday. All groups were fed crushed Nasco frog brittle every weekday. Room and water temperature were maintained at approximately 20° Celsius with a 14:10 photoperiod.

#### Treatment

For the experimental tanks, 1mg of L-thyroxine (Sigma, Lot 111H02 11) was added per liter of water. The L-thyroxine stock was made by adding 0.010g (Mettler Toledo PB303-D Delta Range) of L-thyroxine to 5mL of 1%NaOH (Fisher Scientific, Lot 896729) to create a 2mg/ml solution. Experimental groups were then treated with 1.5mL of the L-thyroxine suspended in 1%NaOH, resulting in a final water concentration of 1 mg/L. Experimental frogs were treated with L-thyroxine stock for 21 days. Control tanks were treated with 1.5mL of 1%NaOH for 21 days. Both control and experimental animals were treated (either with NaOH

or thyroxine) at every water change. After 21 days of treatment, the control and experimental groups resided in untreated water for 21 additional days.

#### Data Collection

Each individual frog was placed in a 250ml plastic bottle with 3mL of untreated dechlorinated tap water. First, each frog was massed using the Mettler College 1300 scale. After massing, the bottles were fitted with atmospheric oxygen gas sensors (Vernier, Beaverton, Oregon). Next, the change in oxygen percentage was measured over a fifteen-minute time interval using Vernier LabPro Oxygen Sensor (Beaverton, Oregon) and LoggerPro software. The oxygen gas sensors were set to take one reading per second for 900 seconds (15 minutes). Two control subgroups and two experimental subgroups were measured each day, resulting in each subgroup being measured every other day. A maximum of 4 frogs were measured at a time.

#### Data Analysis

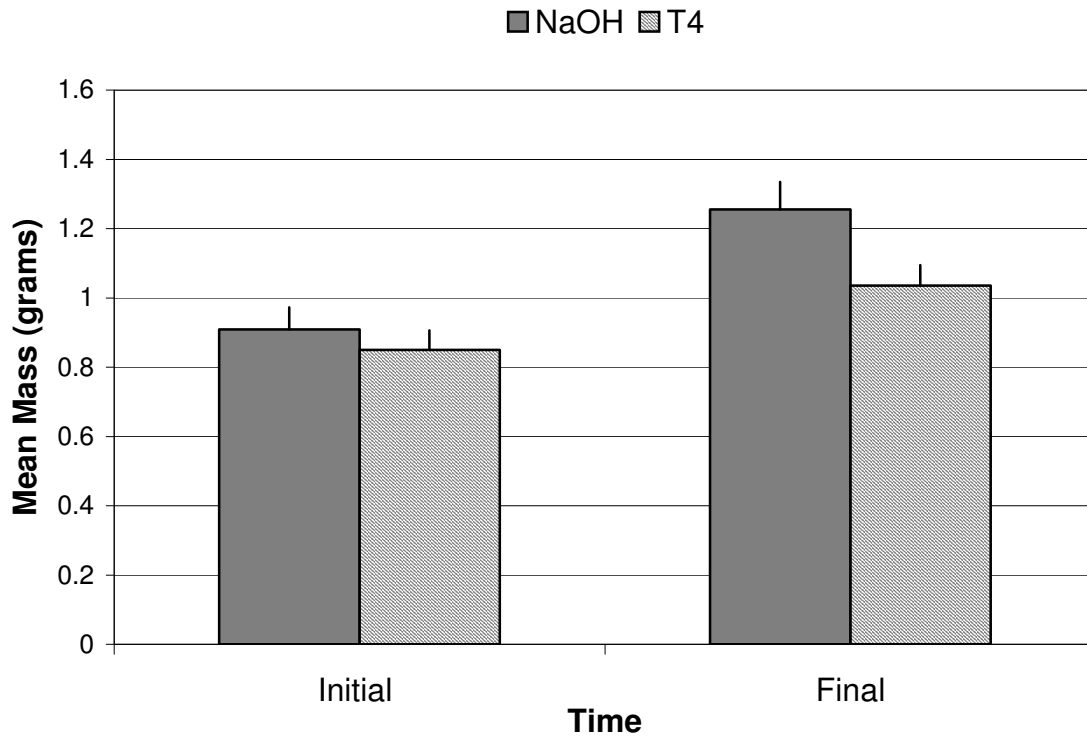
From the raw data, the amount of oxygen (milliliters) consumed per minute was determined. This was converted into metabolic rate by multiplying the results by the omnivore metabolic rate factor, 4.825 Calories/1000ml oxygen consumed. These results were converted from Calories/minute to calories/minute. The result was the metabolic rate per minute, which was converted into the metabolic rate per hour by multiplying by 60. Mass specific metabolic rate per hour was calculated by dividing the metabolic rate per hour by the mass of the frog in grams. The daily mean and standard error was calculated for both metabolic rate and mass specific metabolic rate. Both the mean metabolic rate and mean mass specific metabolic rate were analyzed with regression statistics for days 1-21, 22-42, and 1-42. Change in mass was analyzed using a *t*-test assuming equal variance.

## CHAPTER III

### RESULTS

Figure 3 shows the difference in mass between the thyroxine treated and NaOH control groups at the beginning and at the end of the experiment. Initially, there was no significant difference between the control and thyroxine treated ( $p=0.49$ ). A significant difference was noted for both initial versus final control mass ( $p=0.01$ ) and initial versus final thyroxine treated ( $p=0.03$ ). However, there was no significant difference between the final control and the final thyroxine treated ( $p=0.16$ ).

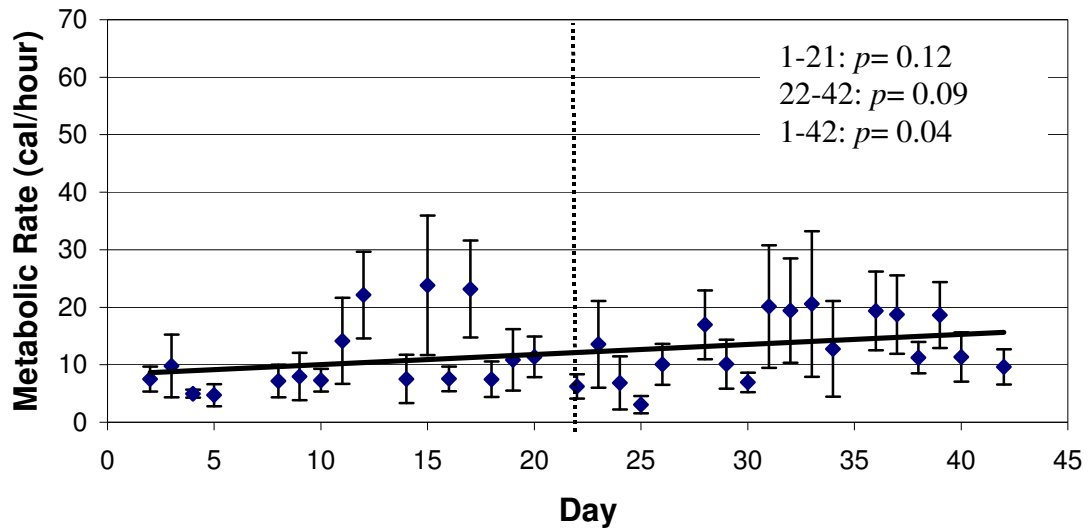
The change in mean metabolic rate over time with the corresponding  $p$ -values is represented in Figure 4. Over the entire experimental period the thyroxine treated exhibited a significant increase in whole body metabolic rate. Though, the increase was not restricted to the treatment period as demonstrated by a  $p$ -value of 0.12 for the treatment period. There was no significant increase for the control's whole body metabolism ( $p=0.55$ ). Figure 5 represents the change in mass specific metabolic rate over time with corresponding  $p$ -values. For the mass specific metabolic rate, there was no significant increase during any time throughout the experiment in both controls and thyroxine treated.



**Figure 3. Mean initial and final mass (+SE) in grams for both the thyroxine treated and NaOH control frogs.**

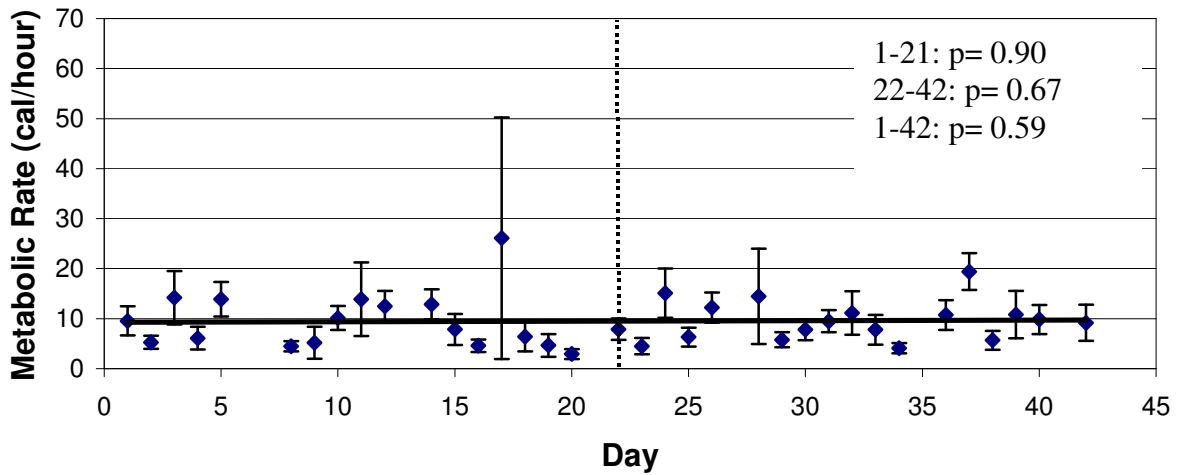
a)

### Thyroxine Treated Mean Metabolic Rate



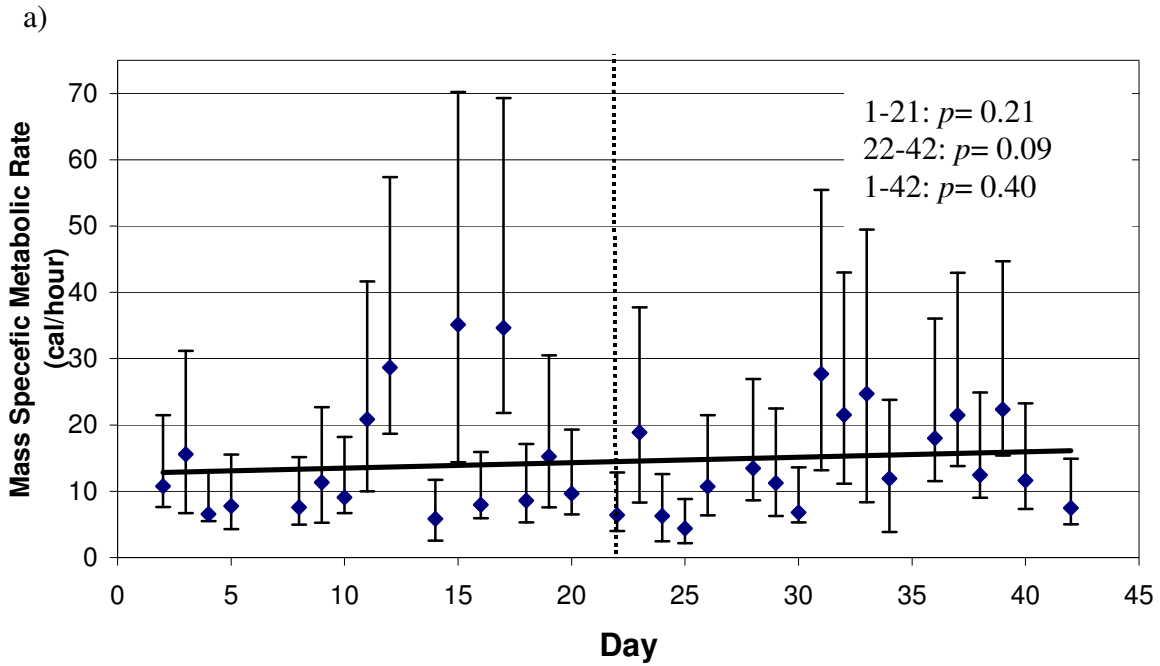
b)

### NaOH Control Mean Metabolic Rate



**Figure 4.** a) Mean metabolic rate of thyroxine treated frogs during the 42-day period with corresponding  $p$ -values. b) Mean metabolic rate of NaOH Control frogs during the 42-day period with corresponding  $p$ -values.

### Thyroxine Treated Mean Mass Specific Metabolic Rate



### NaOH Control Mean Mass Specific Metabolic Rate

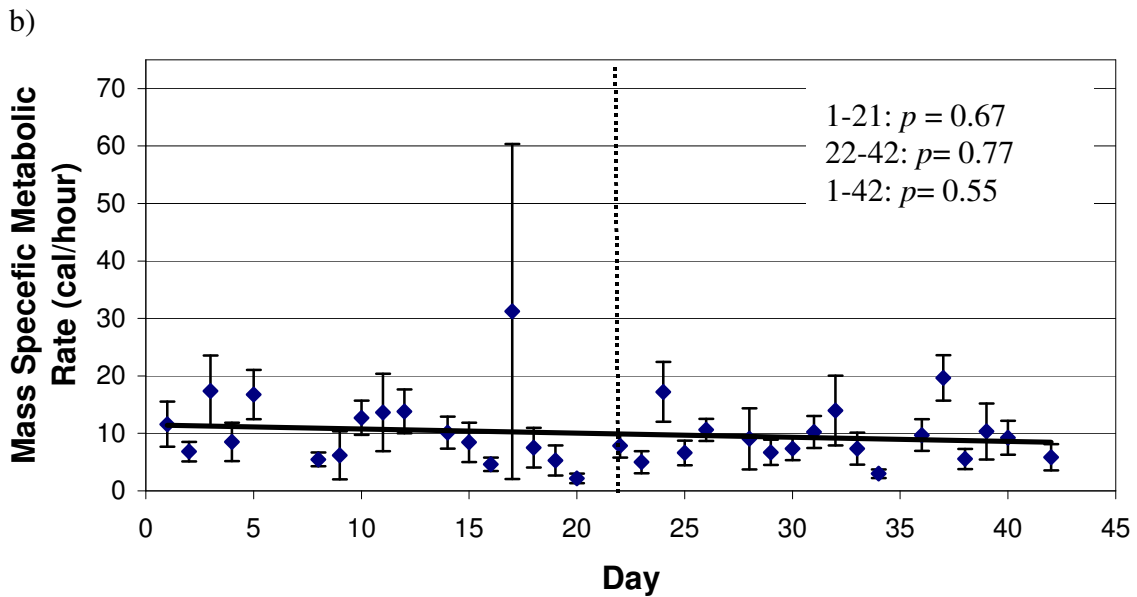


Figure 5.a) Mean mass specific metabolic rate of thyroxine treated frogs during the 42 day period with corresponding  $p$ -values. b) Mean mass specific metabolic rate of NaOH Control frogs during the 42-day period with corresponding  $p$ -values



## CHAPTER IV

### DISCUSSION

The data shows that there was significant increase in whole body metabolic rate over the entire experimental period (42 days) for the thyroxine treated *Xenopus laevis*. However, the metabolic rate increase was not restricted to treatment period. On the other hand, there was no significant increase in whole body metabolic rate of the controls. There was no significant change during any time period for mass specific metabolic rate. In both control and treated frogs, mass significantly increased throughout the experiment. While the control group appeared to gain more mass over time, the difference was not significant.

The whole body result supports the hypothesis that thyroxine treatment would increase the metabolism. However, these results also suggest that metabolic changes were not dependent on time of treatment, which does not support the hypothesis that metabolism would decrease after treatment was halted. This could be due the slow acting nature of thyroid hormones. According to Bentley (1976), thyroid hormones can take several days before achieving maximum effects. Indeed, in rats other thyroid hormones have exhibited extended effects on metabolism. Moreno, Lanni, Lombardi, & Goglia (1997) injected rats with a single dose of either diiodothyronines ( $T_2$ ) or triiodothyronine ( $T_3$ ) and then assessed the rats resting metabolic rate. The resting metabolic rate increased approximately 35% 25-30 hours after the injection of  $T_3$ . The increased rate continued up to 6 days after the injection. After  $T_2$  was injected, the rate increased after only 12 hours and had lowered only after 48 hours. These

results show that thyroid hormones, especially  $T_3$ , can produce prolonged effects on metabolism after initial exposure. Therefore, it is probable that thyroxine would also produce prolonged effects. Rats are endothermic and have much higher energy requirements compared to ectotherms (Randall et al., 2002). Consequently, rats have much higher metabolic rates than *Xenopus*. Because endotherms have elevated metabolic rates, it is likely that more time would be required in *Xenopus* to see the effects diminish. For future studies, an extra time period is needed between measurements to ensure that the previous treatment is no longer affecting the subjects. The delayed action of thyroid hormones could also explain the lack of a significant difference in masses of the groups, as it was originally hypothesized that thyroxine would inhibit weight increase.

Currently, there is no published research that studies the effects of l-thyroxine ( $T_4$ ) on amphibian metabolism, but metabolism has been studied in rats using triiodothyronine ( $T_3$ ), diiodothyronine ( $T_2$ ), and 3,5-di-iodo-L-thyronine (3,5- $T_2$ ). As discussed previously,  $T_2$  and  $T_3$  increase the resting metabolic rate in rats (Moreno et al, 1997). Lanni, Moreno, Lombardi, de Lange, Silvestri, Ragni, Farina, Baccari, Fallahi, Antonelli, and Goglia (2005) tested the metabolic effects of 3,5-diiiodo-L-thyronine on fat storage. Rats were fed a high fat diet and received either no treatment or were administered 3,5-diiiodo-L-thyronine. Rats with 3,5-diiiodo-L-thyronine treatments exhibited a lower body weight and less fat gain when compared to the untreated. The treated rats also demonstrated a higher liver fatty acid oxidation rate, complete loss of liver fat, and a reduction in triglyceride and cholesterol level. These results imply that both  $T_2$  and  $T_3$  play an important role in increasing metabolic rate. Thus, additional research on amphibian metabolism could be completed using these hormones in place of thyroxine.

The significant increase in whole body metabolic rate suggests that thyroxine may have an effect on frog metabolism. Slight changes in the experimental set up and data collection may produce more significant results. Exposing the frogs to thyroxine for a longer period of time and allowing more time for thyroxine withdrawal. Also since *Xenopus* are aquatic, the use of a liquid oxygen probe could produce more realistic and accurate changes in metabolic rate.

Overall, the results suggest that physiological concentrations of thyroxine increases whole animal metabolic rate in *Xenopus laevis*, but has no significant effect on mass specific metabolic rate or body mass increase. Thus, the results did not completely discount the possibility that thyroxine does play a role in *Xenopus* metabolic rate. Further investigations should examine the reason that whole animal metabolic rate is increased, but mass specific is not. Also, further research could be done on the effects of thyroxine independent of metabolism such as changes in liver and thyroid gland histology.

## APPENDIX

MARYVILLE COLLEGE  
Institutional Animal Care & Use Committee (IACUC)  
Animal Research Form

*Provide information after each bold item*

**Student Name:** Angie Castle

**Student Email Address:** angie.castle@maryvillecollege.edu

**Date:** 11/13/2006

**Senior Study Advisor:** Dr. Crain

**Species to be used:** *Xenopus laevis*

**Age of animals:** Hatchlings/ 60 days

**Number of animals in study:** Approximately 100

**Duration of study:** 6 weeks

**Location of animals during the study (building and room) :** Sutton Science 110

**List personnel to call if problems with animals develop:**

Name	Daytime Phone	Nighttime Phone	Emergency No.
Angie Castle	423-802-7428	423-802-7428-	423-802-7428
Dr. Crain	865-981-8238	865-379-1706	

**Husbandry Requirements:** Is anything other than routine care and equipment required?  
YES \_\_\_ No X If "YES", please list below.


**What will happen to the animals at the end of the study? If euthanasia is required, state the methods.**

Euthanasia using MS-222 immersion overdose

*(Do not write below line: For MC IACUC Use)*

Maryville College IACUC Approval Number: 200601

Date Approved: November 21, 2006

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