

THE EFFECTS OF THYROXINE MONOTHERAPY TREATMENT
VERSUS THYROXINE/TRIIODOTHYRONINE COMBINATION
THERAPY: EVIDENCE FROM *XENOPUS LAEVIS* TADPOLES

A Report of a Senior Study

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ABSTRACT

The thyroid is a two-lobed structure that produces hormones necessary to maintain homeostasis in the body. Hypothyroidism is a common disease that is associated with an underactive thyroid gland that does not produce the adequate amounts of thyroid hormones. Two treatment options of oral hormone replacements are available to patients with hypothyroidism. One therapy is the ingestion of the active thyroid hormone thyroxine, and the other is the ingestion of a combination of thyroxine and triiodothyronine. The purpose of this study is to determine if the metamorphic changes associated with *Xenopus laevis* can suggest which therapy option is most effective at treating hypothyroid patients. Tadpoles (n=6 in each group) were exposed to either thyroxine, thyroxine and triiodothyronine, or the vehicle for eight days, and the developmental stage and thyroid histology were measured. The results indicate a significant difference ($p < 0.001$) between the two treatment therapies and the control group when considering follicle diameter, suggesting the hormones have decreased the size of the follicles. Likewise, there is a significant difference ($p < 0.001$) when comparing the epithelial cell height among all three groups, with the combination therapy having the smallest average epithelial cell height ($P < 0.001$). These results suggest the combination therapy has a greater effect on the thyroid gland when considering the follicle size, which directly influences hormone production.

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CHAPTER I

INTRODUCTION

Thyroid

The thyroid gland is a two-lobed, butterfly-shaped structure between the larynx and pericardial sac that is composed of follicles that produce a number of different hormones used for growth, development, and metabolism (Silveira et al. 2013). Within the follicles of the thyroid is a gel-like substance known as colloid that houses the enzymes needed to produce thyroid hormones, as well as the hormones themselves (Hall and Guyton 2011). Thyroid stimulating hormone (TSH), a glycoprotein produced by the anterior pituitary gland, has a primary role in determining the amounts of thyroid hormones present in the circulating blood and intracellular compartments of the body (Li et al. 2014, Soldin et al. 2013, Roelfsema et al. 2013). Production of TSH is under the control of a negative feedback loop between the hypothalamus and the anterior pituitary gland (Segerson et al. 1987). Thyrotropin-releasing hormone (TRH), a tripeptidal hormone, is made by the hypothalamus and is the beginning of the negative feedback loop to produce TSH and the other thyroid hormones including thyroxine (T_4) and triiodothyronine (T_3) (see Figure 1; Roelfsema et al. 2013). After the release of TRH from the hypothalamus, TSH is produced by the anterior pituitary gland, and the synthesis of thyroxine and triiodothyronine begins (Fonseca et al. 2013).

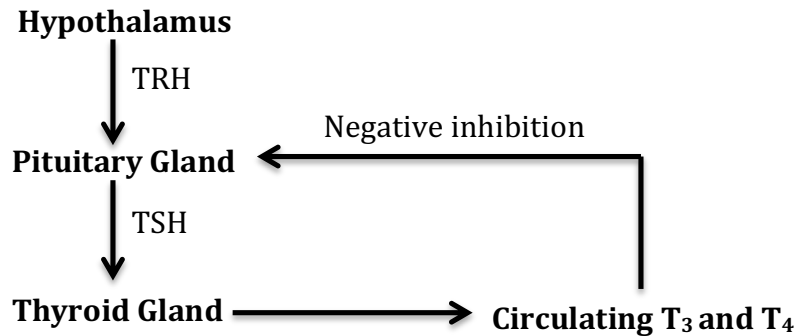


Figure 1. Hypothalamus-pituitary axis negative feedback mechanism.

The production of T_3 and T_4 in the colloid of the thyroid follicles first begins with the transportation of iodides into the thyroid cells by a concentration gradient produced from sodium-potassium ATPase pumps (Hall & Guyton 2011). This is followed by oxidation of iodide ions into a functioning form that is able to bind with tyrosine amino acids produced from the thyroid follicles (Hall & Guyton 2011). When the body is iodine deficient, adequate amounts of hormones are not produced. Thus, it is necessary to ingest iodine in the form of iodides for the human body to function properly. The oxidation of iodide is made possible based on the presence of the enzyme peroxidase that oxidizes the iodide ions into the form of nascent iodine (I^0) or I_3 (Hall & Guyton 2011). Without the presence of peroxidase, the production of T_3 and T_4 is not possible. Once bound to the iodide ions, the tyrosine molecules are coupled to other iodotyrosine molecules to form the major product in the form of T_4 (Hall & Guyton 2011).

T_4 is the major circulating thyroid hormone, and it comprises approximately 93 percent of all of the hormones that are released from the thyroid gland (Hall & Guyton 2011). However, T_4 is not the active form that is needed for the metabolism and growth of the body. The active form of the thyroid hormone is triiodothyronine, and most thyroid hormone

receptors have an affinity for T₃ and not T₄ (Hall & Guyton 2011). T₃ is only approximately 7 percent of the hormones released from the thyroid; so in order to get an adequate amount of the active hormones, tissues must convert the T₄ to T₃ (Hall & Guyton 2011). The conversion of T₄ to T₃ is accomplished in peripheral organs through cleaving of one iodide ion from each thyroxine molecule in the presence of a type 2 deiodinase (D2), which is an intracellular enzyme present along with TSH in the pituitary cells (see Figure 2; Hall & Guyton 2011, Fonseca et al. 2013). Once converted, the active T₃ is absorbed into the tissues and binds to receptor proteins that modify gene transcription (Nikrodhanond et al. 2005). Studies have found that the main function of the inactive form of T₄ molecules that remain after the conversion of T₄ to T₃ may be to bind to mitochondrial cells to increase the activity of the mitochondria to produce adenosine triphosphate (ATP) (Hall & Guyton 2011).

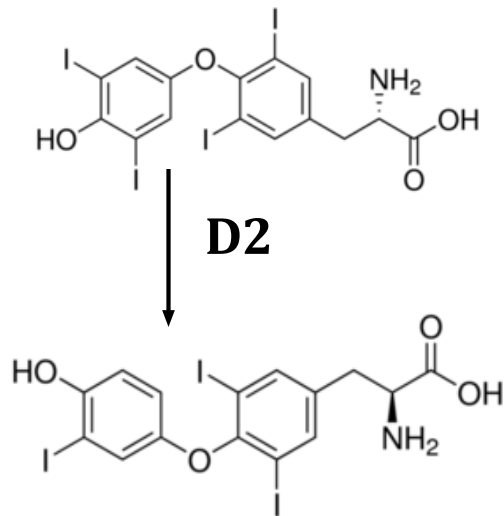


Figure 2. Conversion of Thyroxine (T₄) (Sigma, IRMM468) to Triiodothyronine (T₃) (Sigma, T2877) due to the presence of type II deiodinase (D2).

As previously mentioned, the production of the thyroid hormones requires the proper function of the hypothalamus-pituitary axis negative feedback loop. A reduction in plasma T₄ indicates a deficiency in the iron concentration within the body, causing signals to be sent to

the hypothalamus to begin secreting TRH (Fonseca et al. 2013). The production of TRH activates the pituitary gland to begin releasing TSH followed by the synthesis of the T₃ and T₄ hormones (Fonseca et al. 2013). Increased production of the thyroid hormones triggers a negative feedback stimulation that inhibits the secretion of TSH from the pituitary gland (Hall & Guyton 2011).

Any disruption or malfunction that occurs in the thyroid or hypothalamic pituitary feedback axis can be detrimental to the function of the whole body. An improperly regulated thyroid can cause problems such as energy loss, weight loss or gain, or in extreme cases, miscarriage or coma (National Women’s Health Resource Center 2005). According to the National Graves’ Foundation, approximately 13 million people in the United States are diagnosed with thyroid disorders such as hypothyroidism, hyperthyroidism, or thyroid nodules (National Women’s Health Resource Center 2005). These disorders vary in diagnosis and prognosis (see Table 1).

Table 1. Common thyroid disorders in the United States.

Disorder	Description	Symptoms	Prevalence in United States
Hyperthyroidism	Overactive thyroid gland	Weight loss, irritability, irregular fast heart rate (Silveria et al. 2013)	2.0% over age 55 (Bagchi et al. 1990); 2/1000 pregnancies (Masiukiewicz and Burrow 1999)
Hypothyroidism	Underactive thyroid gland	Fatigue, weight gain, depression, slow heart rate (Silveria et al. 2013)	3.8%-4.6% (Chakera et al. 2013)
Thyroid Nodule	Discrete lesion in thyroid gland	Low TSH levels, swelling in thyroid gland (Cooper et al. 2009)	19%-67% (Cooper et al. 2009)

Hypothyroidism

Hypothyroidism is one of the most prevalent endocrine disorders in the United States and worldwide, and there is a higher prevalence in women than in men (Chakera et al. 2012, Michalopoulou et al. 1998). Previous surveys have shown that the annual incidence of hypothyroidism diagnosis is about 4.1 per 1000 women, and approximately 0.6 per 1000 men (Chakera et al. 2012). Hypothyroidism is a term used to describe an underactive thyroid gland that does not produce the adequate amounts of thyroid hormones necessary to energize the body at a functioning level (National Women's Health Resource Center 2005). There are multiple subcategories of hypothyroidism including primary hypothyroidism, subclinical hypothyroidism, and central hypothyroidism (see Table 2).

Table 2. Most common categories of hypothyroidism and hormone levels associated with each.

Diagnosis	Cause	TSH levels	T₄ levels
Primary hypothyroidism	Autoimmune thyroiditis	↑ TSH (Chakera et al. 2012)	↓ T ₄ (Chakera et al. 2012)
Subclinical hypothyroidism	Abnormal lipid metabolism (Imaizumi et al. 2013)	↑ TSH (Chakera et al. 2012)	Normal T ₄ (Chakera et al. 2012)
Central hypothyroidism	Mutation in pituitary transcription-factor genes (Roelfsema et al. 2013)	↓ TSH (Roelfsema et al. 2013)	Normal T ₄

In healthy adults, normal levels of TSH range from 0.4-4.0 μ U/mL (Michalopoulou et al. 1998). As seen in table 2, the diagnosis of hypothyroidism is based on the levels of serum TSH, as well as the levels of T₄ in the blood (Chakera et al. 2012). Normal levels of T₄ range from 12.0-15.4 pmol/L (Gullo et al. 2011). According to Chakera et al. (2012), primary

hypothyroidism is the most common type of all hypothyroid disorders. Primary hypothyroidism is diagnosed when the TSH levels in the blood are higher than the normal range while the levels of T₄ are below that of the normal range (Chakera et al. 2012). The cause of primary hypothyroidism is due to autoimmune thyroiditis (Chakera et al. 2012). Like all autoimmune diseases, autoimmune thyroiditis is a form of the body attacking itself. Autoimmune thyroiditis is classified by the presence of thyroid autoantibodies in the serum that attack host tissue that eventually may cause the formation of a goiter, which is an enlarged thyroid gland (Dayan and Daniels 1996). Once a patient is diagnosed with hypothyroidism, a lifelong treatment is necessary to regain normal thyroid function. No cure currently exists for hypothyroidism, but with a continuous treatment, patients can regain a euthyroid state to maintain a normal lifestyle.

Treatment for Hypothyroidism

Several treatment options exist for hypothyroid patients to regain a euthyroid state and improve quality of life. Studies have shown that the most successful of these treatments is to use a hormone replacement therapy (Chakera et al. 2012). Older studies experimented with the idea of using a subcutaneous injection of sheep thyroid, but later discovered that the most preferred option is to orally ingest synthetic hormones (Chakera et al. 2012). There are two types of oral synthetic hormone replacement therapies that are utilized by clinicians. These two therapies include the replacement of only T₄ hormones, while the second option is to replace both T₄ and T₃ in a combination therapy. The topic of which therapy is more effective is very controversial due to both having positive and negative effects.

According to Chakera and colleagues (2012), the most successful therapy option to treat hypothyroidism is the T₄ monotherapy. This monotherapy supplies the patient with a dosage of levothyroxine to replace the under-produced thyroxine in the body. The treatment requires a daily dosage due to the short half-life (7 days) of levothyroxine (Chakera et al. 2012). Dosage of levothyroxine is based on body weight of the patient, and it is safe to take approximately 1.6 µg/kg/day (Roos et al. 2005). Once treatment begins, studies have shown that it takes approximately 4 weeks for the TSH and serum T₄ levels to regain normalcy (Roos et al. 2005). The daily dosage of the hormone should be taken on an empty stomach and at one hour before the intake of food to prevent the medication being absorbed by food (Chakera et al. 2012 and Eldresi et al. 2001). Some drawbacks of the therapy include that many medications and foods can cause an unwanted reaction with the levothyroxine; for instance, the drug effects of Warfarin are enhanced by levothyroxine, while the drug effects of Propranolol are decreased when taken with levothyroxine (Chakera et al. 2012). Multiple studies have shown, however, that patients on levothyroxine monotherapy do not reach the desired quality of life, despite the fact that their TSH levels reach the normal range (Escobar-Morreale et al. 2015, Chakera et al. 2012). This dissatisfaction may be due to the assumption that the ingestion of levothyroxine alone is allowing the body to convert enough of the levothyroxine into the active form of T₃ in peripheral tissues, which may not be the case (Escobar-Morreale et al. 2015).

In response to reduced satisfaction with levothyroxine monotherapy, researchers have tested a combination therapy to replace both T₃ and T₄ in the body with synthetic hormones. This option is offered to those hypothyroid patients that feel as though the T₄ monotherapy is either not working or giving them a poorer quality of life (Chakera et al. 2012). The common

name of the combination therapy is referred to as Armour® thyroid, and it prescribes the patient with a T₄ to T₃ ratio of 4:1 (Chakera et al. 2012). However, this differs than the natural T₄ to T₃ ratio in the human body, which is actually 14:1 (Escobar-Morreale et al. 2015 and Chakera et al. 2012). Escobar-Morreale and his colleagues (2015) determined through a study done with thyroidectomized rats that the “ideal” thyroid hormone replacement therapy should contain a ratio of 14:1 to mimic the human hormone secretion. One drawback of this combination therapy is that it may cause an inconsistent fluctuation in T₃ levels, which could have adverse effects on patients causing them to fluctuate between an increased and decreased quality of life (Chakera et al. 2012). Figure 3 shows an algorithm for the treatment and management of hypothyroidism.

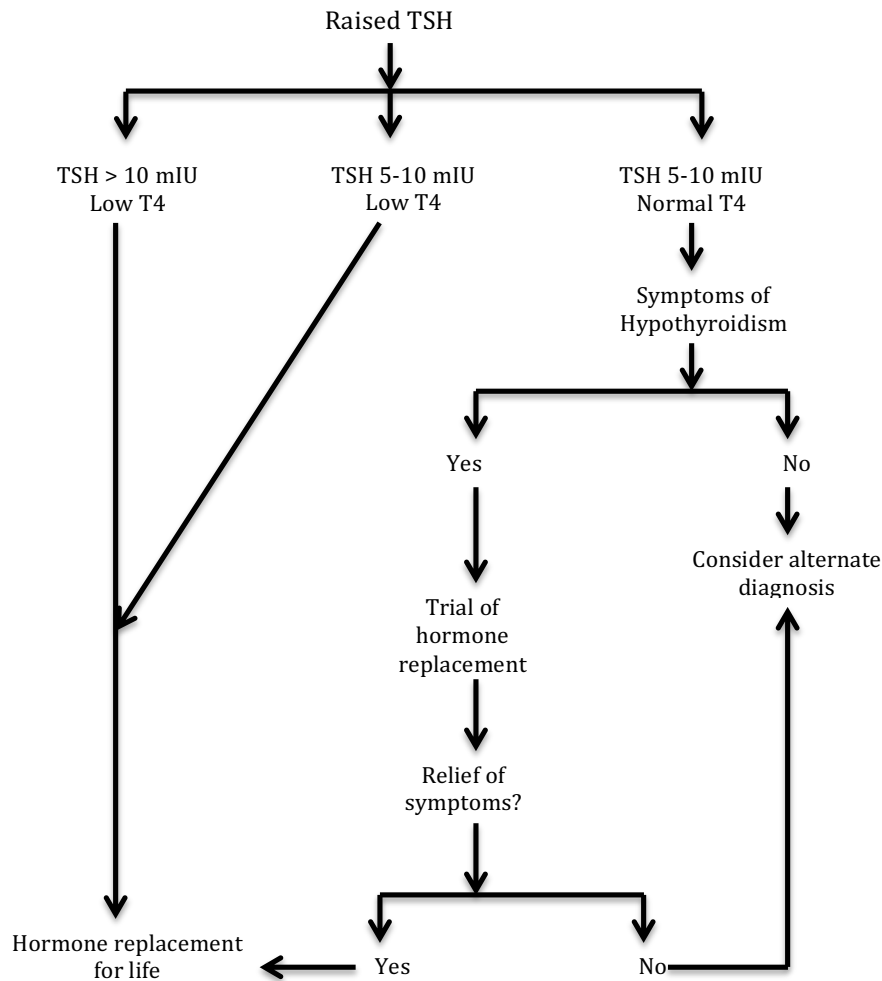


Figure 3. Algorithm for hormone replacement based on TSH and T4 levels (modified from Chakera et al. 2012). (mIU= milli-international unit).

Further research is required to determine the effectiveness of the two therapies compared to each other and also to determine which therapy is the best option to treat hypothyroid patients. This study aims to test the efficacy of the two therapies using *Xenopus laevis* tadpoles as a model organism.

Amphibian as Model Organism

Amphibians have been used as model organisms for decades due to the fact that they are similar to humans at the organizational and functional level (Holmes 1954). For this

study in particular, *Xenopus laevis* tadpoles are an excellent model because of the major importance of their thyroid and the hormones produced in order to complete a successful metamorphosis. Much like humans, amphibians produce thyroxine that is converted to triiodothyronine in the presence of D2 (Duellman and Trueb 1986). A feedback loop between the thyroid, hypothalamus, and anterior pituitary gland also exists, and it is a critical part of the metamorphosis of the organism (Huang et al. 2001). According to Etkin (1968), in early metamorphosis the rise in T₃ and T₄ is part of a positive feedback loop, but when the metamorphosis reaches climax the feedback control changes to negative.

Throughout development and metamorphic changes of vertebrate organisms, the thyroid produces different amounts of thyroid hormones. In *Xenopus laevis*, at metamorphic stage 54 the first secretions of T₃ and T₄ begin, causing the hind limbs begin to appear (Kawahara et al. 1991). According to Kawahara (1991), with the increased exposure to the thyroid hormones, *X. laevis* has a metamorphic climax at stage 59 to 62. The climax is followed by a major decrease in hormone secretion at stage 66 (Kawahara et al. 1991). The sequence of metamorphic changes for *X. laevis* can be seen in Table 3.

Table 3. Metamorphic changes associated with *Xenopus laevis* metamorphic stages (modified from Nieuwkoop and Faber 1994).

Stage	Description
40-47	Gills present, hindlimb bud beginning to show
48	Forelimb bud first visible, thyroid gland with 4-6 lobes
49-50	Forelimb bud oval shaped, hindlimb bud longer, formation of thyroid follicles
51-53	Tentacles longer, Forelimb and hindlimb in paddle stage
54-56	All four fingers and five toes visible
57-60	Fingers stretched out, angle of elbow 90°, formation of septum, tentacles beginning to shrivel, opening of gill chambers
61-65	Head narrower, adult skin, tentacles disappearing, tail disappearing
66 (Climax)	Tail no longer visible from ventral side, mature frog

Along with gross morphological changes, histological changes also take place as the secretion of the thyroid hormones increase. The thyroid gland itself increases in size during larval development, caused by a proliferation of follicle cells and the epithelial cells of the thyroid morph into columnar cells (Duellman and Trueb 1986). The rate of secretion of the thyroid hormones during *Xenopus laevis* metamorphosis is shown in Table 4.

Table 4. Hormone levels during metamorphosis of *Xenopus laevis* (modified from Duellman and Trueb 1986).

Hormone	Premetamorphosis	Early metamorphosis	Late metamorphosis	Climax
TSH	Low	Increasing	High	High
T ₃ and T ₄ Secretion	Low	High	High	High
T ₃ and T ₄ plasma levels	Low	Low	High	Low

Research Question

This study is being conducted to determine: (1) if *Xenopus laevis* can be used as a model to examine the two common therapies used to treat hypothyroidism, either T₄ monotherapy or T₃/T₄ combination therapy and (2) the efficacy of each of these drug treatments as indicated by tadpole metamorphosis and *Xenopus* thyroid histology. Ultimately, the goal of this study is to determine if *Xenopus laevis* can be used to suggest which therapy option is the most effective at treating hypothyroid patients.

CHAPTER II

MATERIALS AND METHODS

TREATMENT 1

Specimen Care

Xenopus laevis tadpoles (n=100) at metamorphic stage 40-47 were obtained from Nasco (enasco.com). Animal husbandry and experimental protocols with these tadpoles were approved by the Maryville College IUCAC (see Appendix A). Six, 1-gallon fish tanks were cleaned and prepared with 3 L of tap water dechlorinated with Top Fin® Tap Water Aquarium Dechlorinator (5mL for 5 gallons). The tadpoles were randomly separated and placed into the fish tanks with 15 tadpoles in each tank. Nasco frog brittle (44% protein, 6% crude fat, 2% crude fiber, and 15% ash) was used to feed the tadpoles every weekday, and a 100% water change was completed every other day.

Dosage

The fish tanks were randomly separated into two experimental groups and one control group, and the aquariums were labeled accordingly. Two aquariums were treated with T₄ monotherapy, two aquariums were treated with T₃/T₄ combination therapy, and two aquariums were the control group that received 1% NaOH.

A 1% NaOH stock solution was made by adding 1.08g NaOH pellets to 108mL of distilled water. To prepare the T₄ levothyroxine monotherapy stock solution, 0.1g of L-

thyroxine (Sigma, T2376) was added to 50mL of 1% NaOH and vortexed. 1500 μ L of L-thyroxine stock solution was added to each group chosen to receive T₄ monotherapy. The final concentration given to the *X. laevis* tadpoles was 1mg/L. This dosage was given to the tadpoles every other day following the 100% water change.

To prepare the T₃/T₄ combination therapy stock solution, 0.006g 3,3',5-Triiodo-L-thyronine sodium salt (Sigma, T6397) and 0.094g L-thyroxine (Sigma, T2376) was added to 50mL of 1% NaOH. Each aquarium chosen to receive the combination therapy was given 1500 μ L of the T₃/T₄ stock solution every other day following a 100% water change. The final concentration placed in the two tanks was 1mg/L. Control tanks received 1500 μ L of 1% NaOH every other day following a 100% water change.

Euthanasia

Prior to receiving any treatment, three *X. laevis* tadpoles at metamorphic stage 40-47 were euthanized for preparation of histology examination. Tricaine methanesulfonate (MS-222; 400mg/L) was used to anesthetize the tadpoles. The tadpoles were then weighed for body mass and preserved using Bouin's fixative.

TREATMENT 2

Specimen Care

As a result of 100% mortality, a second treatment was conducted. *X. laevis* tadpoles (n=20, stage 57-59) were obtained from Nasco (enasco.com). Three, 10-gallon fish tanks were cleaned and prepared with 24 L of dechlorinated tap water as previously described in treatment 1. The tadpoles were randomly divided, and six tadpoles were placed in each tank.

Nasco frog brittle was fed to the frogs each day, and there was a 100% water change before every treatment was given.

Dosage

Prior to receiving any treatment, one *X. laevis* tadpole at stage 58 was euthanized for preparation of histology examination. Tricaine methanesulfonate (400mg/L MS-222) was used to anesthetize the tadpole. The tadpole was then weighed for body mass and preserved using Bouin's fixative.

The aquarium tanks were randomly divided into one control group and two experimental groups and labeled accordingly. One aquarium was treated with T₄ monotherapy, one aquarium was treated with T₃/T₄ combination therapy, and one aquarium was the control group that received 1% NaOH. Using the previously prepared stock solution from treatment 1 (0.006g T₃, 0.094g T₄, 50mL NaOH), 3000μL was added to the tank selected to receive T₄ monotherapy. This resulted in a final concentration of 0.25mg/L of T₄ added to the tank. For the tank receiving T₃/T₄ combination therapy, 3000μL of the stock solution previously prepared containing a 14 to 1 ratio of levothyroxine and triiodothyronine was added to the aquarium. Likewise, the aquariums determined as control groups received 3000μL of 1% NaOH. Each treatment was given every three days following a 100% water change of the tank.

Euthanasia

Throughout the experiment, tadpoles were staged according to Nieuwkoop and Faber (1994) and weighed on a Mettler balance. After 8 days of exposure to the hormone treatments, all of the tadpoles were euthanized in Tricaine methanesulfonate (400mg/L,

Sigma), the thoracic cavity was opened, and it was then placed in Bouin's fixative (Sigma, HT10132).

Histology Preparation and Analysis

After seven days of fixation in Bouin's fixative, the tissues were cleared in 70% ethanol and placed in paraffin wax blocks, and histology slides were made according to Humason's 5th edition (Presnell et al. 1997). The wax was cut into 12 μ m sections, floated in water, and placed on glass slides. The slides were stained using eosin and hematoxylin, and they were analyzed under a microscope. The total number of follicles present in each frog thyroid was counted, and epithelial cell height (n=40 for each individual) and follicle diameter (n=8 for each individual) were measured. SPSS software was utilized to conduct an analysis of variance (ANOVA) test, and if significant, a Tukey's post hoc analysis was conducted.

CHAPTER III

RESULTS

TREATMENT 1

After three days of exposure (2 treatments given), the dosage of 1mg/mL resulted in a 100 percent mortality rate of all tadpoles in the aquariums given the monotherapy and combination therapy treatments. In the control group three days after exposure, there were 5 dead tadpoles resulting in a 17% mortality rate. Thus, a lower dose treatment (Treatment 2) was initiated.

TREATMENT 2

The mortality rate in treatment 2 was much lower than that of treatment 1. A total of four tadpoles receiving T₄ monotherapy treatment died over the eight-day treatment. Three tadpoles receiving T₃/T₄ combination therapy died, and two control tadpoles died over the duration of the experiment. The numbers of deaths after days of exposure are shown in Figure 4.

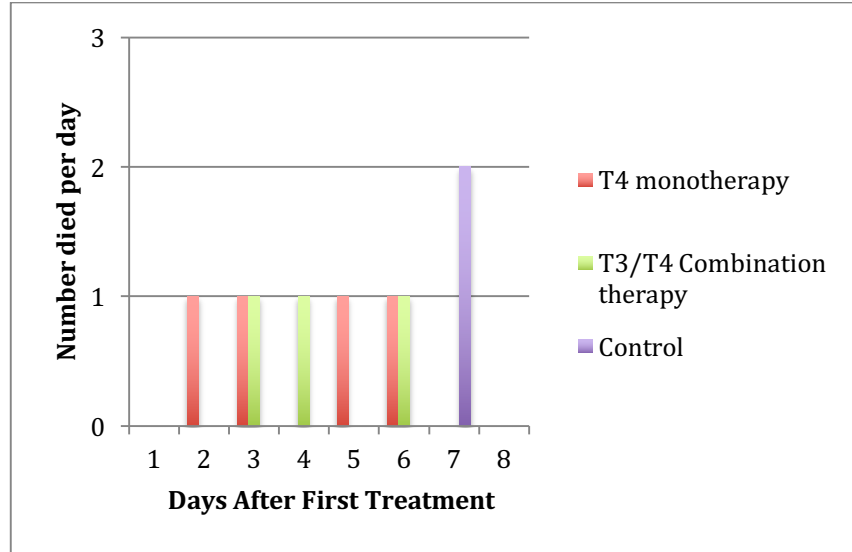


Figure 4. Number of tadpoles dead per day after first exposure to treatment. Number of T₄ monotherapy tadpoles dead: n=4; Number of T₃/T₄ tadpoles dead: n=3; Number of control tadpoles dead: n=2.

The tadpoles in each group were staged and weighed prior to any treatment. Each tadpole was individually staged and weighed during the duration of the experiment on days 6, 7, and 8. The results of the ANOVA test indicate no significant difference in the average weights and stages for each treatment and control group ($p=0.66$; $p=0.62$). The average stages and weights of the tadpoles in each group are shown in Figure 5.

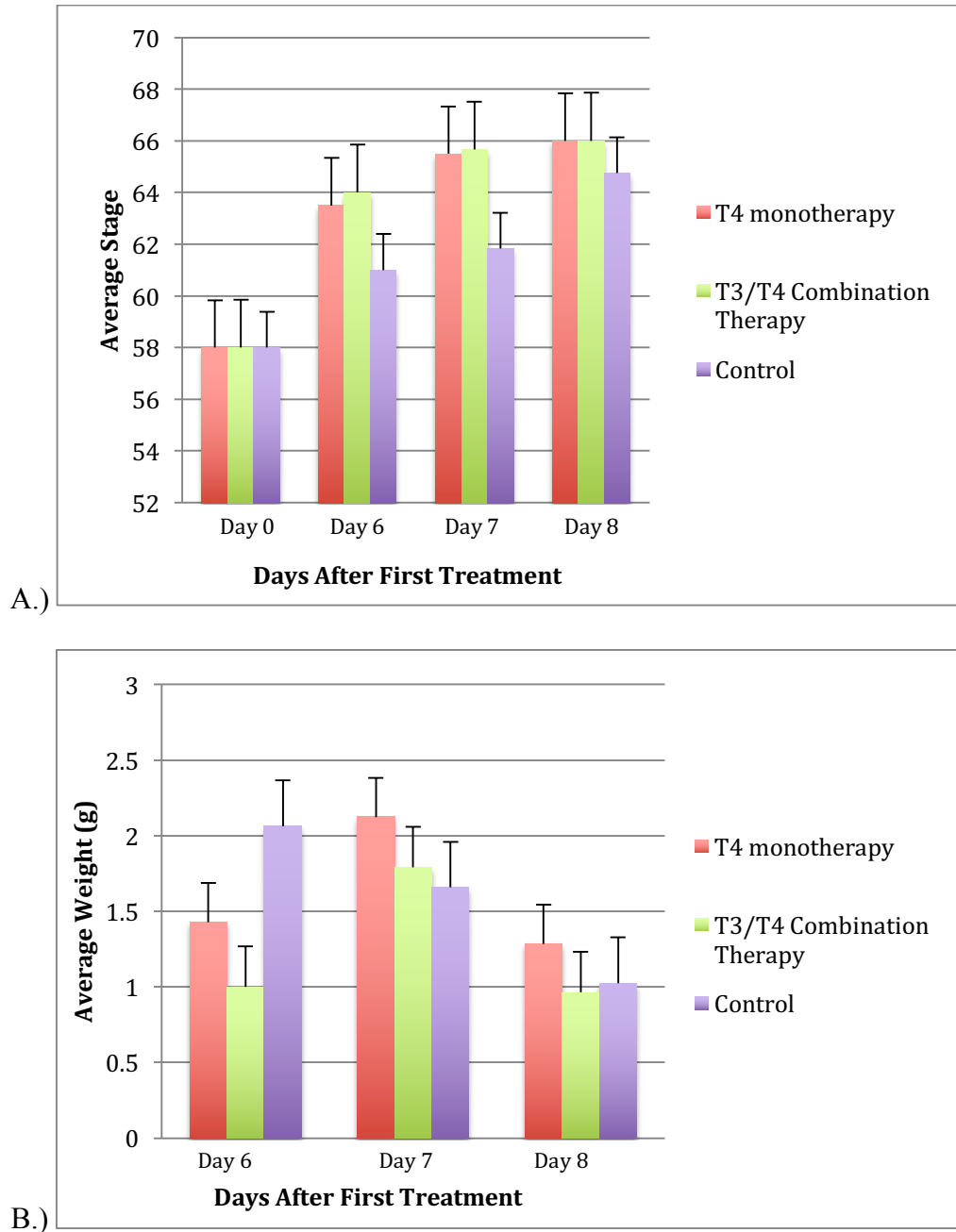


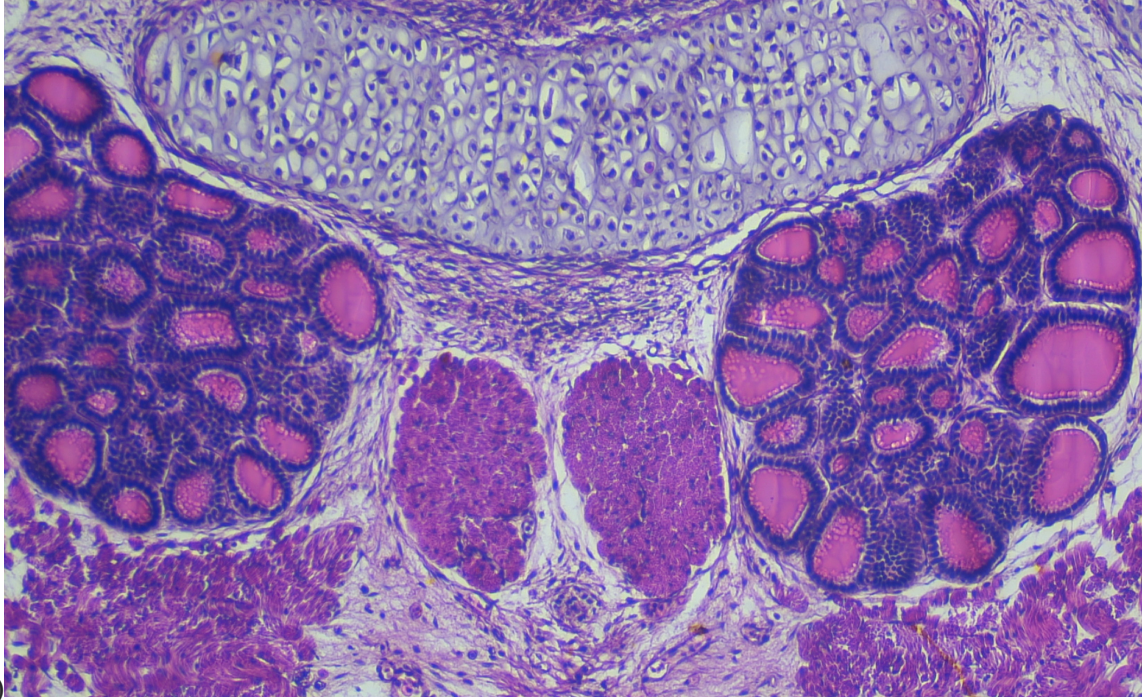
Figure 5. Average (+1 SE) A.) stage and B.) weight of tadpoles after receiving first treatment of T₄ monotherapy, T₃/T₄ combination therapy, or NaOH (control group) according to Nieuwkoop and Faber (1994).

The prepared histology slides were compared and analyzed. The follicle contains colloid (pink stain), and it is surrounded by simple cuboidal epithelium (purple stained nuclei). Figure 6 shows the components measured for each thyroid for each treatment and

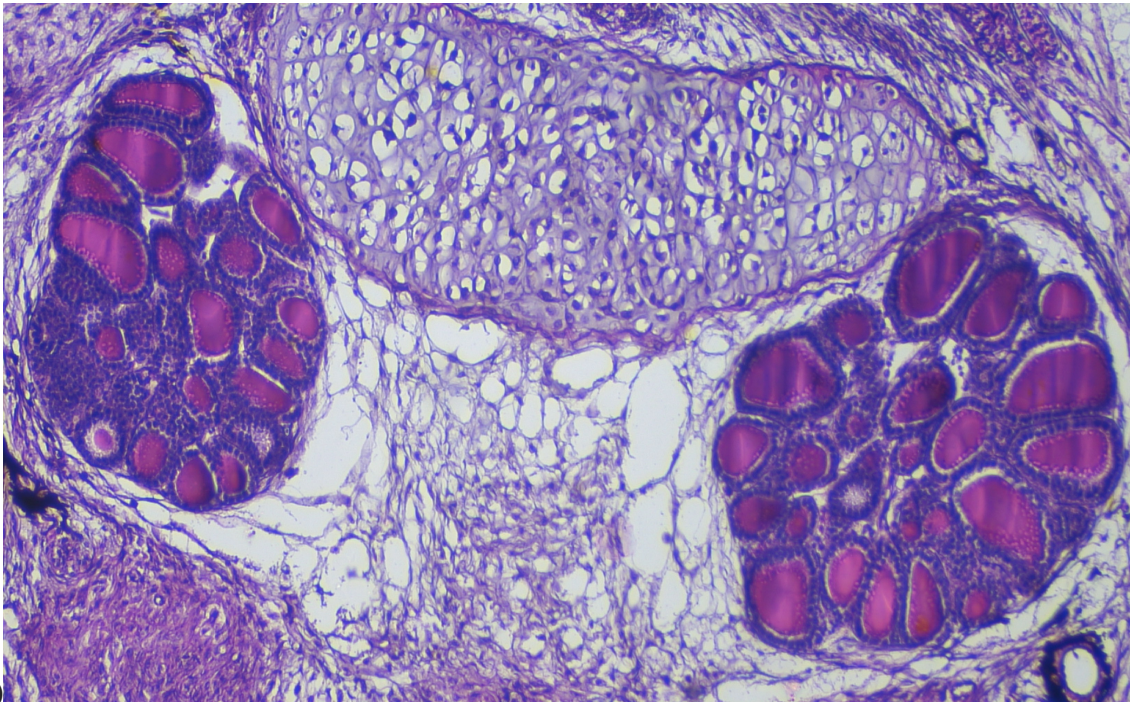
control group, and Figure 7 shows a representative thyroid from one frog from each treatment or control group.



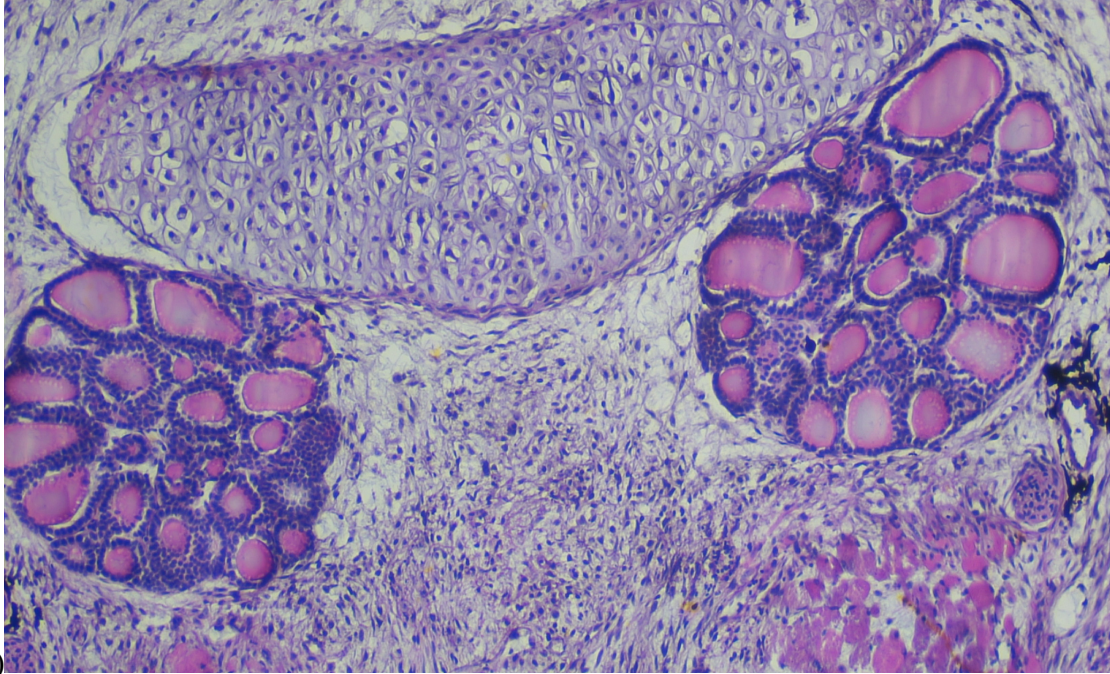
Figure 6. Labeled thyroid follicle showing the area used for measuring follicle diameter and epithelial cell height. (100x mag).



A.)



B.)



C.)

Figure 7. Cross-section images of thyroid follicles from a.) control group, b.) T₄ monotherapy treatment group, and c.) T₃/T₄ combination therapy treatment group. (40x mag).

Each follicle was counted in all frogs in each treatment or control group. Whereas the average number of follicles was slightly greater in the T₄ monotherapy group (30) compared to the control group (24.8) and T₃/T₄ combination therapy group (25.5), this was not significantly different ($p= 0.30$; see Figure 8)).

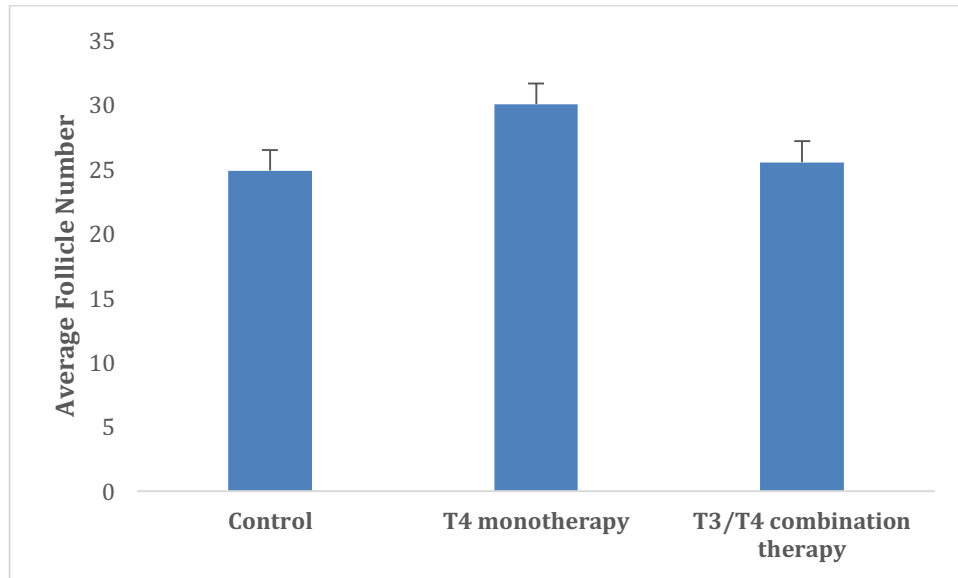


Figure 8. Average number of follicles (+1 SE) for each treatment and control group. Control: n=3; T4 monotherapy: n=2; T3/T4 combination therapy: n=3.

The average follicle diameter for each treatment and control group was determined by measuring the diameters (n=8) of each frog for each treatment and control group. The average follicle diameter was different in frogs treated with T4 monotherapy (48.6 μ m) and T3/T4 combination therapy (48.4 μ m) and the control group (59.2 μ m; p=0.0009). The average follicle diameters are shown in Figure 9.

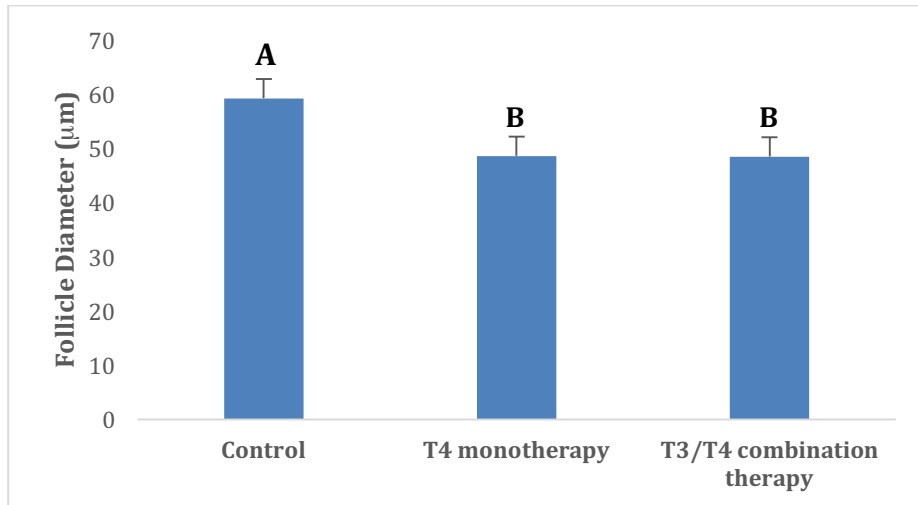


Figure 9. Average diameter of follicles (+1 SE) for each treatment and control group. Control: n=3; T4 monotherapy: n=2; T3/T4 combination therapy: n=3. Tukey's post hoc analysis suggests A and B are significantly different (p=.002; p=.007).

Similarly, for each control and treatment group, the epithelial cell height was measured and compared. The height of 40 epithelial cells from each frog for each treatment group was measured. There was a significant difference (p<.001) in the epithelial cell height between T₄ monotherapy (8.6μm), T₃/T₄ combination therapy (6.9μm), and the control group (11.4μm; See Figure 10).

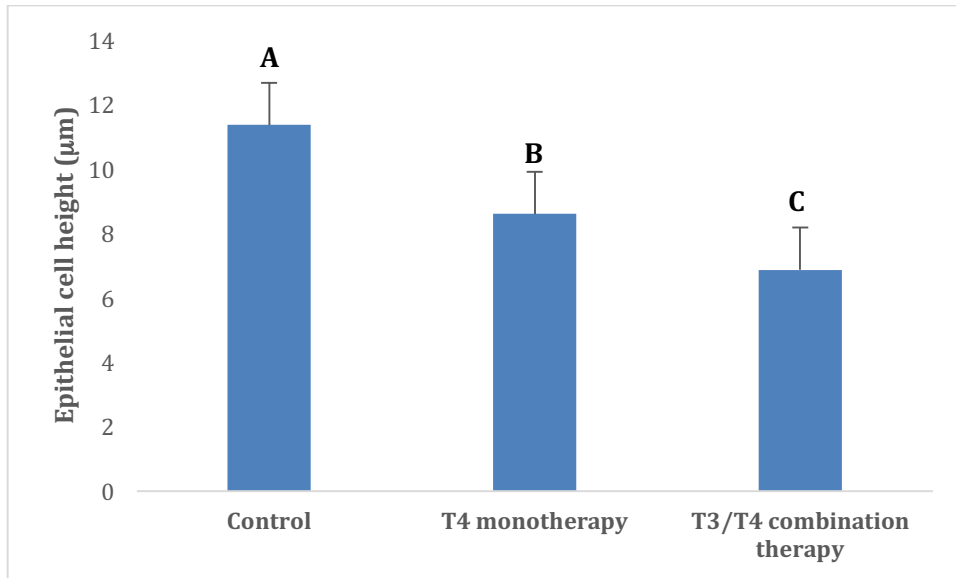


Figure 10. Average height of epithelial cells (+1 SE) surrounding follicles for frogs in each treatment group. Control: n=3; T4 monotherapy: n=2; T3/T4 combination therapy: n=3. Tukey's post hoc analysis suggests A is significantly different from B ($p<.001$), B is different from C ($p<.001$), and A is different from C ($p<.001$).

CHAPTER IV

DISCUSSION

The results show no significant difference in the survival rate of the three different treatment options (T_4 monotherapy, T_3/T_4 combination therapy, and no hormone replacement). Likewise, there is no difference in the weights and metamorphic progression of the three groups. However, the measurements taken of the individual thyroids of each frog do indicate that the different hormone replacements have different effects on the growth and development of the thyroid. The follicle diameter in both groups given a hormone replacement decreased significantly from the control group receiving no hormonal replacement. This indicates that more colloid is present in the thyroid of frogs that did not receive any additional hormones via consumption. According to Barbier et al. (1976), the size of the colloid present in the follicle of a thyroid indicates the scale to which TSH is produced. A larger colloid signifies a greater production of TSH, whereas a smaller colloid limits the amount of TSH that can be produced (Barbier et. al 1976). With this information, the results suggest that both therapies do work to reduce the amount of TSH produced, and those frogs given no hormone treatment have a greater amount of TSH present than those that were given a hormone treatment. It can be assumed that with a lesser amount of TSH present, more circulating T_3 and T_4 is present due to the negative feedback system. In other

words, if the anterior pituitary recognizes a greater amount of circulating hormones, the production of TSH would be inhibited.

An important function of TSH is to increase the number of thyroid cells and to change the cells from cuboidal epithelium with a shorter height to columnar epithelium (Hall and Guyton 2011). The height of the epithelial cells can determine whether this transformation has occurred or not. The results of this study indicate the hormone replacements have an effect on this transformation, and it is shown through the measurements of the epithelial cell height. However, unlike the measurements of follicle diameter, there were significant differences among all three treatment groups. The epithelial cell height in the group receiving T_4 monotherapy was on average significantly less than the control group. However, the epithelial cell height in the group receiving T_3/T_4 combination therapy was on average much less than the control group as well as the T_4 monotherapy group. These data suggest that the T_3/T_4 combination therapy group has an even smaller amount of TSH produced in the colloid, determining that a greater amount of circulating T_3 and T_4 are present. Thus, in metamorphosing frogs, the T_3/T_4 combination therapy has a greater effect on the development of the thyroid. These data suggest that T_3/T_4 functions better to reduce the amount of TSH produced and to increase circulating T_3 and T_4 .

Previous research on the controversial topic of monotherapy versus combination therapy has been conducted, and Gullo et al. (2011) presented results similar to those discussed above in humans (see Table 5). Gullo and colleagues tested these two treatment therapies by measuring TSH and circulating T_3 and T_4 levels in patients receiving T_4 monotherapy compared to those receiving T_3/T_4 combination therapy. Their results show that patients receiving T_4 monotherapy eventually reach normalcy in their levels of TSH and

circulating T₄. However, some patients were not able to regain the normal levels of circulating T₃ (Gullo et al. 2011). Gullo and colleagues (2011) suggest that this group of patients whom are unsatisfactory with levothyroxine alone benefit from the addition of triiodothyronine to their treatment, but that levothyroxine monotherapy should be the standard for treating those with hypothyroidism.

Another study done testing the effects of the two therapies discovered that patients taking the levothyroxine and triiodothyronine combination therapy benefit neuropsychologically, and that their psychological state was much more improved than those taking T₄ monotherapy (Bunevicius et al. 1999). Within their results, Bunevicius et al. (1999) discovered that the patients treated with the combination therapy had higher circulating T₃ and T₄ levels than those that were given only levothyroxine monotherapy. The increased T₃ levels present appear to have significantly positive effects on treating the low hormone levels in hypothyroid patients, as well as improving overall mental health. Other previous research suggests similar results, and some of these literature results are presented in Table 5.

Table 5. Summarized literature findings on previous research comparing levothyroxine monotherapy to levothyroxine/triiodothyronine combination therapy.

Treatment	Summarized Finding	Reference
Levothyroxine monotherapy vs. Combination therapy	Patients taking combination therapy benefit neuro-psychologically, and they had higher numbers of circulating T ₃ and T ₄	Bunevicius et al. 1999
Levothyroxine monotherapy vs. Combination therapy	Patients reached normal TSH and circulating T ₄ levels; However, many patients were not happy with quality of life and preferred combination treatment.	Gullo et al. (2011)
Levothyroxine monotherapy vs. Combination therapy	Improved quality of life and psychometric functioning; However, no biochemical differences in reaching normal hormone levels than those patients receiving T ₄ monotherapy	Chao et al. (2009)
Levothyroxine monotherapy vs. Combination therapy	No benefits symptomatically of the combination therapy	Grozinsky-Glasberg et al. (2006)

In conclusion, the results of this study indicate through follicle diameter and epithelial cell height in *X. laevis* tadpoles treated with T₄ monotherapy and T₃/T₄ combination therapy that levothyroxine and triiodothyronine combination therapy has a greater effect in lowering TSH levels and increasing circulating T₃ and T₄ levels. In further research, however, these results should be considered while also determining any effects the combination therapy compared to the monotherapy may have on other peripheral organs present in the organism.

In other words, if the T₃/T₄ combination therapy has negative impact on other organs present in the body, the substitution of triiodothyronine for levothyroxine may increase the circulating hormones but have negative long-term effects indicating that levothyroxine monotherapy should be the standard for treating hypothyroidism.

APPENDIX A

**MARYVILLE COLLEGE INSTITUTIONAL ANIMAL CARE & USE COMMITTEE
Application for Use of Vertebrate Animals in Student Research**

Provide information after each bold item

Student Name:
Madison Coker
Student Email Address:
Madison.Coker@my.maryvillecollege.edu
Date:
8 August 2015
Senior Study Advisor:
Dr. Drew Crain
Species to be used:
Xenopus laevis
Age of animals:
Metamorphic Stage 40-47
Number of animals in study:
90
Duration of study:
7 Weeks
Location of animals during the study (building and room):
Sutton 114

List personnel to call if problems with animals develop:

Name	Daytime Phone	Nighttime Phone	Emergency No.
Madison Coker	865-776-8483		865-776-8483
Dr. Drew Crain	865-981-8238		

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

At the end of the study, three frogs from each group will be euthanized with tricaine methanesulfonate (400mg/L MS-222 with 1g/L sodium bicarbonate added as a pH neutralizer). Histology slides will be made of the thyroid gland in each of these tadpoles. The rest of the late metamorphose tadpoles will be used for Bio414: Developmental Biology.

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

Maryville College IACUC Approval Number: 201514

Date Approved: Aug 3, 2015

Signed: _____



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