GENISTEIN EFFECTS ON REPRODUCTIVE AND THYROID HORMONES

A Report of a Senior Study

by

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Date Approved _____, by ______________

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Date Approved_____, by ______________

Editor

ABSTRACT

Genistein, a phytoestrogen component of soy, has become a common replacement for estrogen therapies for post-menopausal women; however, the effects of genistein on the endocrine systems and thyroid hormone pathways are not completely understood in healthy or hypothyroid individuals. Hypothyroid individuals were hypothesize to show greater estrogenic responses and lower thyroid activity when exposed to genistein than healthy individuals. *Xenopus laevis* tadpoles (metamorphic stage 56) were exposed to genistein (10 µM) for 21 days with and without an iodine dietary supplement (0.030 µM) to mimic hypothyroidism. Tail length and weight were measured throughout the experiment and final metamorphic stages and mortality rates were assessed. Histological ovarian samples were analyzed for estrogenic effects on follicular diameter. Both groups exposed to genistein lost significantly ($p=0.003$) more weight than the control tadpoles, with the ample iodine group losing the most and having the greatest reduction in tail length ($p<0.008$). Mortality due to inadvertent starvation differed among groups (Chi-square $p=0.100$): hypothyroid (60%), genistein with iodine (30%), and control (10%). No significant differences existed with respect to metamorphic rate ($p=0.624$) or follicular development. Ovarian development from the bipotential gonad was limited to one individual in both the genistein and iodine group (average follicular diameter 7.2 µm) and in the control group (14.1 µm). Results do not support the hypothesis, although genistein exposure affected the hypothyroid group differently than healthy groups.

Exposure to genistein increased physiological stress, as seen by higher mortality; however, genistein interactions with iodine allow for increased metabolic effects beyond exposure to iodine or genistein alone.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

ACKNOWLEDGEMENTS

I would like to thank the city of Maryville for arranging the iodine analysis of the campus tap water. I would also like to extend my gratitude to Maryville College security for opening the building during the summer so that I could begin my experiment early. Mrs. Debbie Threadgill always made sure I had access to all the equipment I needed, and for that I am very appreciative. Special thanks to Erin French for her support and helping hand, but most of all, I would like to thank Dr. Drew Crain for all of the time, guidance, and wisdom that he gave when supervising my study.

CHAPTER I

INTRODUCTION

Soy consumption in developed nations has increased as people support healthy natural foods and supplements and oppose genetically modified foods or chemically engineered medications. Soymilk is a lactose-free alternative for lactose-intolerant individuals and vegans, soy supplements have been used in place of estrogen therapies for post-menopausal women, and soy consumption has been shown decrease prostate cancer in high-risk males (Kurahashi et al., 2007). According to the American Soybean Association (2007), the U.S. exported 29.9 million metric tons of soy beans in 2006, a number that has been steadily increasing since 1988 when only 14 million metric tons were being exported.

Because soy is considered a natural product, its use is not regulated by the Food and Drug Administration, but this does not mean it has no negative consequences in individuals that consume it. Soy contains phytoestrogens that affect the endocrine systems of both males and females, and soy has been shown to interact with the thyroid hormone pathways. The endocrine systems of all vertebrates are highly complex and all interactions among hormones are not fully understood. Currently, much research has been conducted on how the thyroid hormone pathway interacts with the reproductive system. Because soy has effects on both of these systems, studying the interactions of soy in animals may help to elucidate unknown relationships of the endocrine system.

Thyroid Hormone Axis

In all vertebrates, the thyroid hormones increase transcription of many genes through a cascade of hormonal pathways, and this increased transcription elevates metabolism (Figure 1). Thyrotropin releasing hormone from the hypothalamus stimulates the release of thyroid stimulating hormone (TSH) from the anterior pituitary thyrotropes, and the TSH binds to receptors on the thyroid gland. The thyroid gland responds by producing more thyroxine (T_4) and triiodothyronine (T_3), which require iodine for synthesis. The thyroid secretes T_3 and T_4 into the bloodstream, and both thyroid hormones bind to proteins because the molecules are extremely insoluble. T_4 enters cells and becomes deiodinated into T_3, which is the metabolically active thyroid hormone.

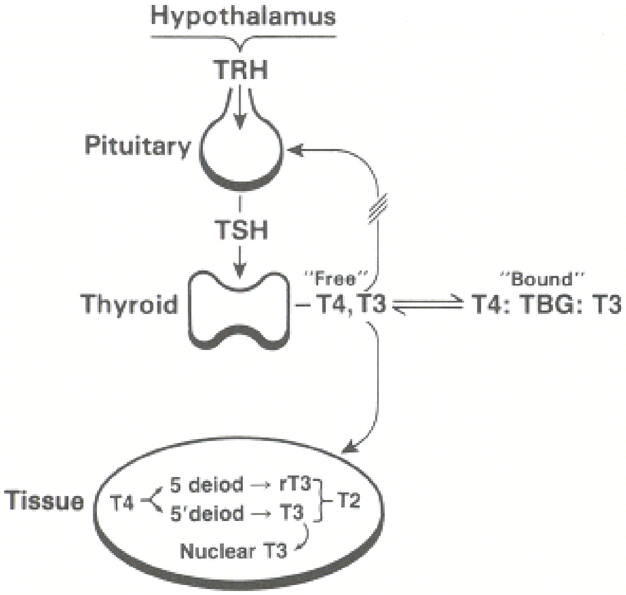


Figure 1: Thyroid hormone cascade (Walker et al., 1990, pg. 142)

Estrogen Hormone Axis

Estrogen production is also the result of multiple hormonal pathways, and estrogenic compounds regulate secondary sexual characteristics in female vertebrates. In a human female, the circulating concentrations of estradiol-17β vary depending on the day of the monthly menstrual cycle. Estrogen production is stimulated by the release of gonadatropin-releasing hormone from the hypothalamus, which further stimulates the anterior pituitary to release follicle stimulating hormone (FSH). At the ovaries, FSH promotes development of follicles prior to ovulation and the remaining corpus luteum after ovulation; both follicles and corpus luteum secrete estrogenic hormones.

Influence of the Thyroid Axis on Reproduction

The thyroid and estrogen hormone pathways are seemingly unrelated, but do have interactions. Although the glands that secrete the hormones are spatially separated, they are both regulated via hypothalamic and anterior pituitary input. Much research has been dedicated recently to the relationships between the thyroid and reproductive systems. Many of these studies focus on the affects thyroid hormones have on the menstrual cycles and reproductive systems of females. The menstrual cycle, which is controlled by estrogen and progesterone concentrations, can be affected by T_4 to produce abnormalities and infertility (Poppe et al., 2007). The mechanism of this effect is not fully known, but in order for thyroid hormones to affect any cell there must be a specific receptor on that cell. Thyroid hormone receptors have been found on ovarian surface epithelium (Rae et al., 2007), and these receptors illicit a stimulatory response on cellular functions when treated with T_4. This molecular model explains how thyroid hormones can affect the reproductive systems.

Another example of the affect of thyroid hormones on female reproduction is illustrated in women with thyroid autoimmune disorder (TAID). Infertile women have a higher frequency of the disorder than the general population (Poppe et al., 2002). There is also a significant increase in the frequency of endometriosis in women with TAID. Therapy with thyroid hormones treats the symptoms of TAID and improves menstrual irregularities and fertility. This shows a correlational relationship exists between thyroid hormones and reproductive abnormalities, although it does not indicate which system has a causative effect on the other, if one exists. However, all of these studies are suggesting that abnormal levels of thyroid hormones may have an adverse affect on reproductive capabilities.

Influence of Estrogens on the Thyroid Axis

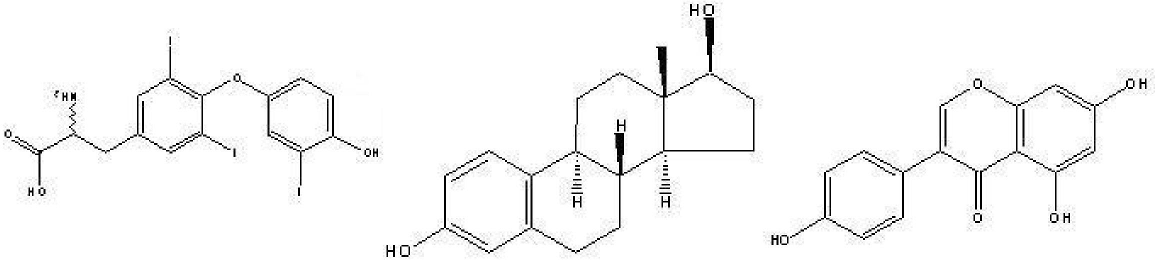
As well as questioning how the thyroid hormones affect the reproductive system, researchers are also exploring the affects of reproductive hormones on the thyroid. Just as the thyroid hormones must bind to thyroid hormone receptors on the ovary to elicit effects, estrogen also must bind to specific estrogen receptors on the thyroid to exert an effect. While estrogen receptors have not yet been found in the thyroid tissues, they have been found in the endothelium of thyroid-associated vascular tissues (Ceresini et al., 2006). This creates an effective pathway for estrogen to interact with the release of thyroid hormone. Oral estrogen replacement therapy in post-menopausal women and post-operational transsexuals increases the amount of circulating thyroxine-binding globulin (TBG) (Bisschop et al., 2006). Increased circulating TBG concentrations decrease the amount of thyroid hormone available because a greater concentration of thyroid hormones would bind to the proteins, which can have adverse effects on metabolism. Estrogen therapy decreases the circulating T_3 levels; however, the other thyroid and thyroid stimulating hormones are not changed (Lima et al., 2006). Supraphysiological levels of estrogen promote an increase in iodide uptake by the thyroid gland. Estrogen interactions with normal thyroid processes also display that there is a close relationship between estrogen and the thyroid hormones.

Estrogenic compounds also have influences on the thyroid and reproductive systems. Red wine contains phytoestrogens that increase TSH and T_3 concentrations (Bottner et al., 2006), whereas estrogens have been found to decrease T_3 (Lima et al., 2006). Bisphenol A (BPA) is also a well-known estrogenic compound that has agonistic effects on estrogen receptors (Crain et al., 2007). BPA also acts as an antagonist on thyroid receptors by inhibiting transcription that thyroid hormones activate (Moriyama et al., 2002). In addition, BPA activates transcription of genes that thyroid hormones suppress. This shows that estrogen and estrogen-like compounds often influence thyroid hormone mechanisms.

Genistein (4',5,7-Trihydroxyisoflavone) is an estrogenic compound found in soy-based foods, and is gaining acceptance as a natural alternative for post-menopausal estrogen replacement therapy (Ryokkynen et al., 2005). Genistein’s relatively long half-life in the human body (8.36 hours; Wantanabe et al., 1998), and the compound’s ability to accumulate in body stores (Penza et al., 2007) gives the compound ample time to interact with the endocrine system. Genistein has many estrogenic interactions that are regulated through binding directly to estrogenic receptors (Doerge & Sheehan, 2002) including increasing trabecular bone volume and decreasing the resorption of bone that is a common cause of osteoporosis (Taguchi et al., 2006). High soy intake has also been associated with increased circulating estrogen concentrations in women (Taguchi et al., 2006), and decreased estrogen concentrations in males (Ryokkynen et al., 2005).

Genistein also has direct interactions with thyroid hormones, much in the same manner that estrogen does. Genistein increases circulating thyroglobulin and autoantibodies against thyroid hormones (Milerova et al., 2006), which decreases available thyroid hormones in circulation. Iodine deficiencies, which can cause hypothyroidism, greatly affect genistein's thyroid interactions in two ways. The lack of iodine naturally decreases the available thyroid hormones by hampering their synthesis (Doerge & Sheehan, 2002). Secondly, genistein has the unique ability to replace tyrosine as a substrate for iodination by tyrosine peroxidase (Divi et al., 1997), thus decreasing the amount of iodine that would be otherwise utilized for T_3 and T_4 production. This compounded problem is thought to manifest itself in the form of a goiter, which could lead to misdiagnosis of autoimmune hypothyroidism in individuals that have high soy intake. The ability of genistein to become tri-iodinated may play an important role in interactions with thyroid receptors. However, not all interactions between genistein and thyroid compounds are negative. Genistein intake is an effective therapy used against familial amyloidosis, which is an accumulation of transthyretin fibrils caused by improper binding of thyroxine (Radovic et al., 2006). Genistein properly binds to transthyretin and inhibits fibril formation and thus eradicates symptoms of the disease.

To understand how genistein has the ability to bind to both thyroid and estrogen receptors and binding proteins, it is important to compare the chemical structures of T_3 , estradiol-17β, and genistein (Figure 2). All three molecules have a ring-based structure with hydroxyl side groups that may be important in interaction with the hormonal receptors. As stated previously, genistein also has the ability to be tri-iodinated to more-closely resemble T_3.



Triiodothyronine Estradiol-17β Genistein

Figure 2: Structures of triiodothyronine, estradial-17β, and genistein

Experimental Model

Genistein has both estrogenic and anti-thyriodic effects, but it is unclear if the amount of genistein affecting the thyroid quantitatively alters the ability of genistein to exert estrogenic effects. An effective animal model to examine the ability of genistein to inhibit normal thyroid activity is *Xenopus laevis*. It has been shown that exposure to genistein inhibits tail regression during the final stages of metamorphosis in *Rana catesbeiana* (Ji et al., 2007). The mechanism of tail regression, which requires ample thyroid hormones, is similar in *Xenopus* (Kawahara et al., 1991) and, thus, geinistein-induced tail retention is expected to occur in *Xenopus*. Estrogenic effects can also be measured by quantitatively analyzing ovarian follicular development in *Xenopus* models.

In order to determine if genistein's estrogenic effects are altered when thyroid conditions are different, control and iodine-deficient hypothyroidic *Xenopus* frogs will be exposed to genistein. If genistein exerts its effects on the thyroid system by undergoing iodination, the lack if iodine will decrease the percentage of genistein that affects the thyroid axis and will increase the amount of genistein available to interact with the ovaries. We hypothesize that in the presence of genistein, slower rates of metamorphosis will be present. In addition, it is hypothesized that ovarian follicular development will be increased in *Xenopus* with hypothyriodic conditions. These predictions are illustrated in Figure 3.

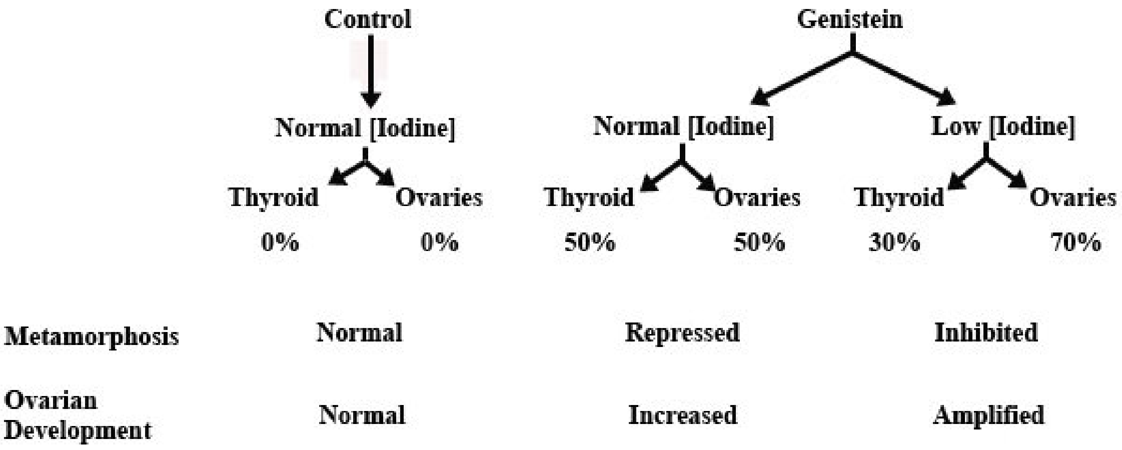


Figure 3: Schematic representation of hypothesized effects of genistein exposure in normal and low iodine concentrations. In individuals with normal levels of iodine it can be predicted that only a portion of genistein (50%) will affect the thyroid via iodination and the remaining genistein (50%) that has not been iodinated will affect the ovaries. As compared to the control, normal iodine concentrations should produce repressed metamorphosis and increased ovarian development. In low iodine concentrations, the thyroid is already inhibited because the thyroid hormones require iodine. There is

expected to be a smaller percentage of genistein (30%) available for iodination, but overall the antithyroidic effect is expected to be greater. Thus, greater than normal percentage of genistein (70%) is expected to be available to exert effects on the ovaries. In this situation metamorphosis should be greatly decreased and perhaps even completely inhibited and ovarian development should be amplified even more than in normal iodine conditions.

CHAPTER II

MATERIALS AND METHODS

Animal Husbandry

Thirty *Xenopus laevis* tadpoles at stage 56 were obtained from Nasco (Fort Atkinson, WI). After dividing the tadpoles into three groups of ten, the tadpoles were staged according to Nieuwkoop and Faber (1994) metamorphic stages, massed, and their tails were measured to assure no significant differences existed between groups. Tadpoles were placed in tanks with aged dechlorinated tap water treated with Fresh Start. The tadpoles were fed Tetrafin© Goldfish Flakes daily and a 100% water change was performed every nine days. A 14:10 photoperiod was maintained with an average water temperature of 20°C.

Experimental Design

The control tank received an iodine supplement, World Organic's Liquid Kelp (2.54 µg of iodine per mL), which added 0.030 µg of iodine per liter of water, not including iodine which may already be present in the tap water. The first experimental tank was given the same iodine supplement and treated with genistein (98%, Sigma Chemical Co. Lot#046K1312) to a final concentration of 10 µM. The final experimental tank was treated with genistein (10 µM) in the absence of an iodine supplement. Tetrafin goldfish flakes were selected as the diet due to the iodine-free, low soy content of these flakes.

Endpoints Measured

Water samples (50 mL) from each of the tanks were collected in polypropylene tubes analyzed by Microbac Laboratories, Inc. (Maryville, TN) to determine the exact concentration of iodine in each tank and in the Maryville City tap water using the American Oil Chemists Society (AOCS) technique of Inductively Coupled Plasma-Mass Spectrometry.

The length of each tadpole tail (from the end tip of the dark peritoneum at the base of the body to the tip of the tail) was measured using Vernier calipers and each tadpole was massed after removing excess water at the start of the experiment, during every water change, and at the end of the experiment. Each tadpole was also examined morphologically to determine its metamorphic stage at the beginning and at the end of the experiment. Animals were observed daily, with any deaths being documented.

Histology

After 3 weeks of treatment, the surviving tadpoles were anesthetized in 0.05% MS222 (ethyl-2-aminobenzoate in ethane sulfate). Gonads and attached kidneys were removed under a dissection scope from anesthetized or deceased tadpoles. The tissues were placed in Bouin’s fixative for 3 days, and then transferred to 75% ethanol to clear excess fixative for 4 days. The tissue samples were dehydrated using a series of increasing concentrations of ethanol starting with 2 hours at 80%, 1.5 hours at 95%, 1 hour in 100%, and ending with 1 hour in fresh 100% ethanol. Next, the tissues were placed in CitriSolv, a non-toxic clearing agent, for one hour and placed in fresh CitriSolv for an additional hour. After the tissues were cleared, they were infiltrated with paraffin wax under a vacuum for 1 hour at 12, 15, 21, and 25 pounds per square inch. The samples were embedded in a wax block, which was subsequently trimmed and sectioned using a microtone to a thickness of 12 micrometers. The sections were mounted on glass slides which dried over night on a slide warmer. A standard Hematoxylin and Eosin stain was performed on the dried slides and a coverslip was added with Permount. The slides were analyzed in order to sex each tadpole, and in the case of an ovary the diameters of ten follicles were measured for each individual.

Statistical Analyses

Data was imported to Microsoft Excel and a one-way ANOVA ($p < 0.05$) was performed to determine if any significant differences existed among groups in respect to tail length, metamorphic development, and mass before, during, and after treatment. A Fisher PLSD post-hoc test was performed in Statview on tail regression data. Chi-square tests ($p < 0.50$) were also performed using Excel to analyze differences for mortality and percent weight loss.

CHAPTER III

RESULTS

AOCS analysis of the water samples showed that no sample had greater than 50.0 µg/L of iodine. The average percent weight loss (Figure 4a) and tail length in millimeters (Figure 4b) of each experimental group was determined on day 0, 8, and 19. Significant differences were found in average percent weight loss between the control group and the groups that were exposed to genistein on day 8 and day 19 (p=0.003 and 0.157). Both tadpole groups exposed to genistein lost significantly more weight than the control tadpoles. On day 8 a significant difference in tail length could be seen (ANOVA p=0.003). The tadpoles exposed to genistein in the presence of iodine had the greatest reduction in tail length (p<0.008), while the control and iodine deficient genistein exposed tadpole groups had the lowest reduction in tail length. The average metamorphic stage of each experimental group was determined at the beginning of the experiment and again on day 19 (Figure 4c), and no significant differences existed among the groups at the end of the experiment (p=0.624).

Deaths occurred in all tanks after the data collection on day 8 and on day 19 (Figure 5). There is a statistical difference in mortality among groups on Day 8 (p=0.100). The greatest mortality occurred in the iodine deficient genistein group (60%), then the genistein with ample iodine group (30%), and the control group had the lowest mortality (10%). Analysis of histological slides showed that the control group contained two individuals with distinct ovarian follicular development (Figure 6a). The genistein with iodine group contained one individual with clear follicular development (Figure 6b), and the iodine deficient genistein group lacked individuals with any clear gonadal development. The average follicular diameter of the genistein group was 7.2 µm at metamorphic stage 62.5, while the control group had an average of 14.1 µm at metamorphic stage 61.5.

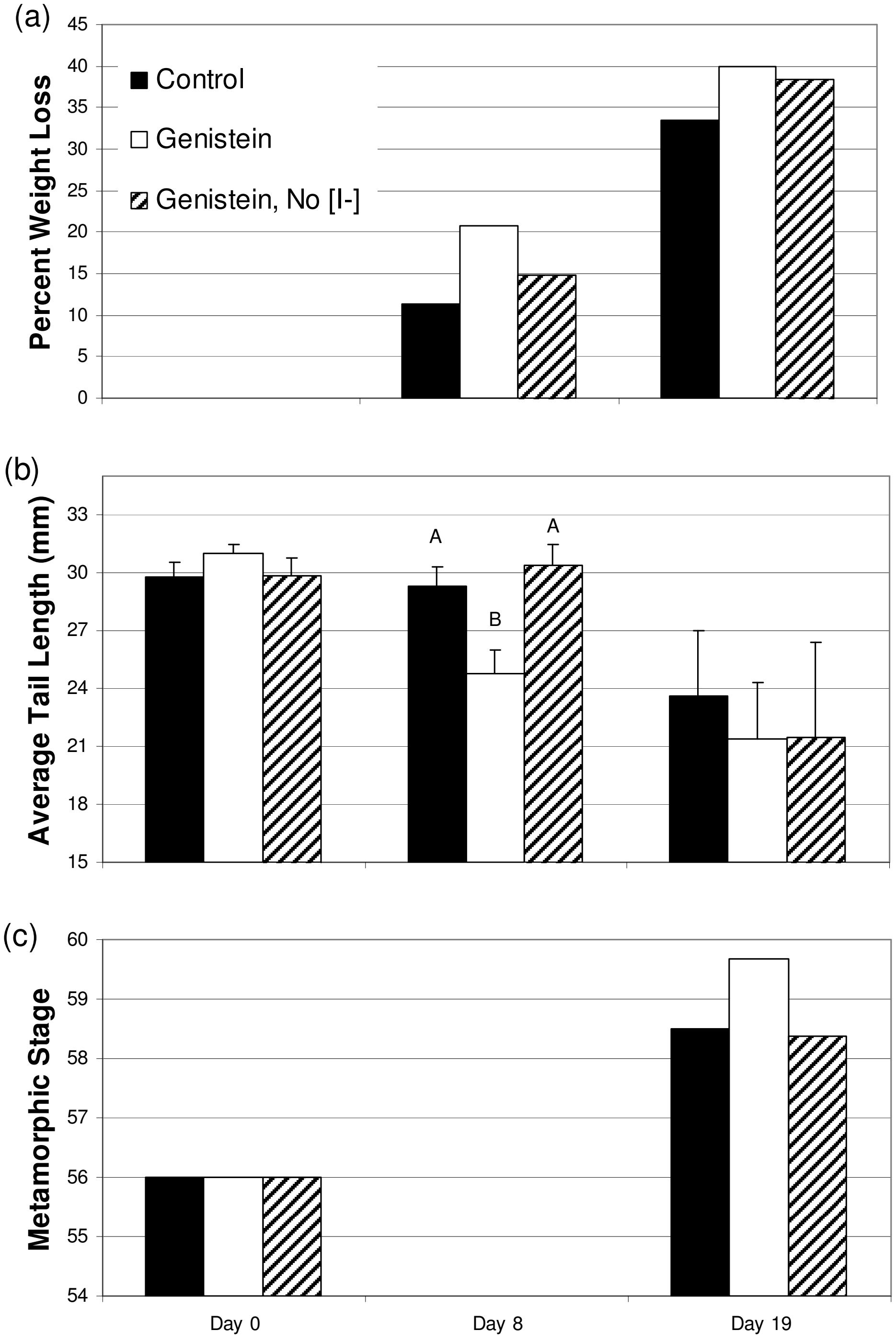


Figure 4: Average (a) percent weight loss, (b) tail length (+1SE), and (c) metamorphic stage (+1SE) of each experimental group on Day 0, Day 8, and Day 19. Different superscripts (b) represent significance at $p<0.008$.

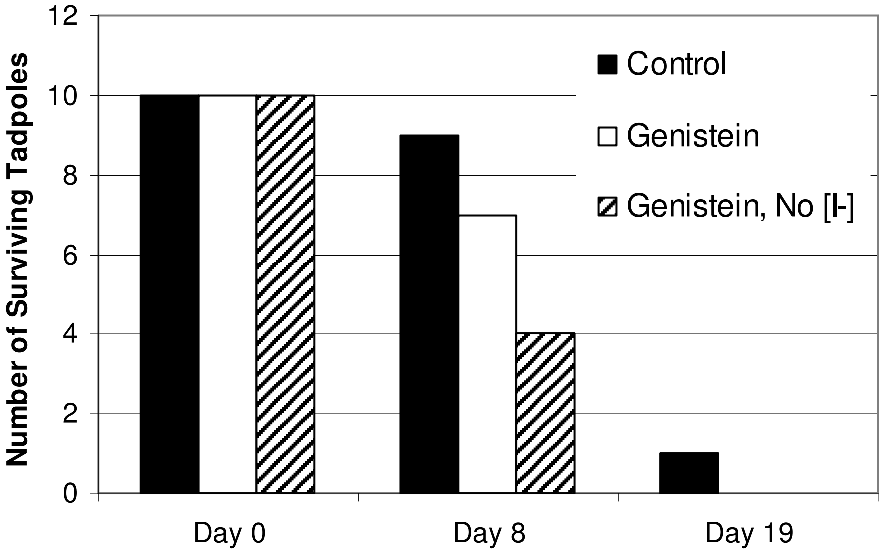


Figure 5: Number of surviving tadpoles from each experimental group on Day 0, Day 8, and Day 19.

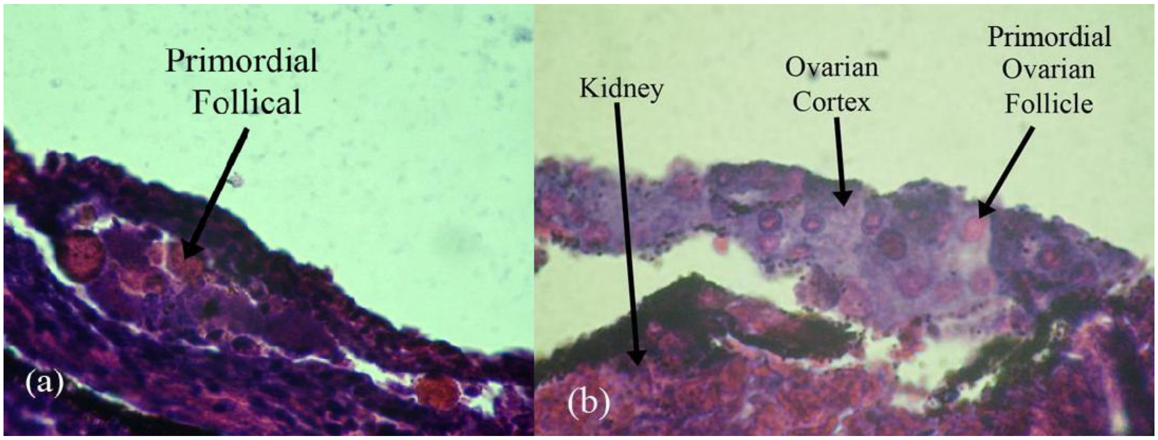


Figure 6: Photograph of *Xenopus laevis* ovary with associated primordial follicles at 1000x from (a) control group and (b) genistein group.

CHAPTER IV

DISCUSSION AND CONCLUSION

Genistein exposure was hypothesized to decrease metamorphic rate and increase ovarian development, with the effects being more prominent in iodine deficient tadpoles. The results do not support this hypothesis. Increased percent weight loss and tail resorption in the iodine sufficient genistein-exposed group shows genistein having an apparent agonistic effect on the thyroid hormone pathway, as the tadpoles expressed symptoms of an increased metabolism. While not significant, the genistein-exposed tadpoles exhibited a higher average metamorphic stage on Day 19, supporting the suggestion that genistein is acting as a thyroid hormone agonist. Although exposure to pharmacological levels of genistein (100µM) is known to inhibit metamorphosis in *Rana* species (Ji et al., 2007), physiological levels (10 µM) of genistein exposure in iodine deficient tadpoles showed no significant differences with respect to tail length or weight loss compared to the control group in *Xenopus laevis.* These effects could be explained by genistein's ability to be iodinated, but there is no evidence that the iodination of genistein would allow genistein to agonistically bind with thyroid receptors. Genistein's antithyroid effects seem to be limited to decreasing the efficiency of thyroid peroxidase and a goiter-causing build up of iodinated genistein in the thyroid gland (Doerge & Sheehan, 2002). This activity does not alter the circulating thyroid hormones in healthy individuals, although it may alter the amount of thyroid hormone therapy needed in hypothyroid patients or patients with a dietary iodine deficiency due to a slight absorption inhibition (Messina & Redmond, 2006). This evidence points to an interaction between genistein and iodine that somehow increases metabolism, without necessarily having direct involvement with thyroid hormone receptors.

Post-mortem dissection revealed limited intestinal content in a majority of the tadpoles, explaining the high mortality from each tank after the stress of water changes. The tadpoles did not eat their iodine-free goldfish diet, and thus suffered the effects of starvation. The higher mortality rates in the genistein exposed groups show that genistein added to this dietary stress, with the highest mortality rates being in the iodine-deficient group.

While the mechanism through which genistein increases physiological stress is unknown, it is proposed that genistein exposure reduced energy stores in the tadpoles. Besides increasing mortality, starvation would also decrease circulating amino acids. The lack of dietary tryptophan would halt thyroid hormone synthesis, which would explain why no differences could be seen between the control group and the iodine deficient group with respect to tail length or weight loss. In the environment with ample iodine, the presence of genistein reversed these effects and increased weight loss and tail resorption was seen in this group, which is illustrated in Figure 7. This may be explained by a recent finding that genistein can reduce overall body weight by reducing glycogen and adipose tissue stores and decreasing circulating insulin (Szkudelska & Nogowski, 2007), but if so this effect is inhibited by an iodine-deficient diet.

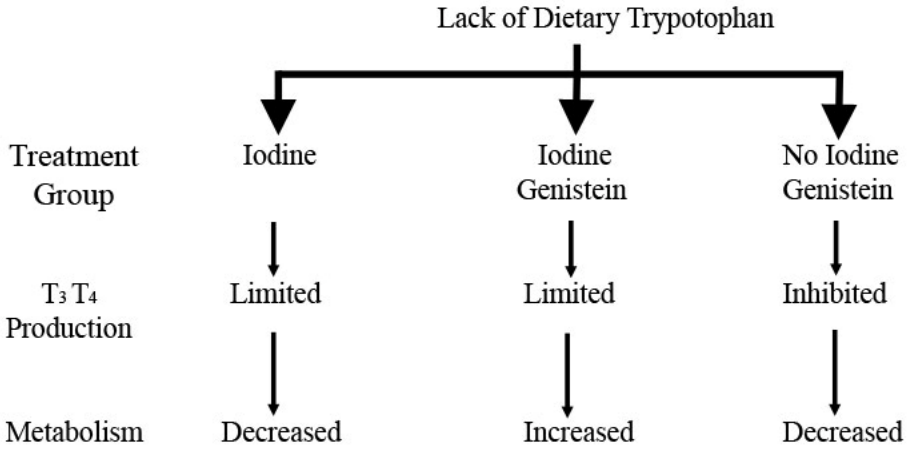


Figure 7: Schematic representation of results.

Very few ovaries were obtained from tadpoles to compare ovarian histology, and the lack of significant histological evidence for the estrogenic effects of genistein is due to the early mortality of the tadpoles. Seminiferous tubule development begins at stage 59 and oocyte development at stage 56, but these are not fully formed structures until the tadpole has reached stage 66 (Nieuwkoop & Faber, 1994, pg. 138). The average metamorphic stage for all tadpoles was stage 59. Starvation would also have had an effect on endogenous estrogen production. The lack of dietary cholesterol and the decreasing ability to synthesize cholesterol from acetyl coenzyme A would have greatly hindered estrogen synthesis from cholesterols. The young metamorphic stage and inhibited estrogen synthesis explains the inability to determine sex in a majority of the tadpoles. Indeed, the only ovarian follicles to be found were in tadpoles at stages 61.5 and 62.5 in groups with sufficient iodine. Although individuals existed at this stage in the iodine deficient group, no ovarian follicles were found. The genistein exposed ovarian follicles unexpectedly had a smaller average diameter (7.1µm) than those found in the control group (14.2µm), although the genistein exposed individual was at a higher metamorphic stage (62.5). This suggests that iodination of genistein may inhibit its ability to interact as a phytoestrogen, and future studies should examine the possibility.

In order to determine if these results are due to the combined lack of tryptophan and varying levels of iodine, further research would need to be conducted with a diet deficient in both tryptophan and iodine or a replication of this experiment without the effects of starvation. Varying concentrations of genistein and iodine should also be attempted to verify the reproducibility of these findings. In addition, more sensitive methods of iodine concentration assessment are also needed to quantify low iodine solutions. Because no antithyroid activities were found in this experiment, other components of the soy bean may be necessary to illicit a response. Future studies should concentrate on combinatorial studies of various soy isoflavones and proteins. In order to determine if iodine deficiency increases estrogenic effects of genistein, tadpoles should be exposed before natural ovarian follicular development has occurred to assess the effects on follicular development. Subsequent groups should be exposed to genistein after initial follicular development to determine effects on follicular growth. This experiment demonstrates the importance of dietary choices in animal husbandry because the inadvertent starvation may have altered the outcome of the original experiment. Powdered egg is an alternative soy and iodine free diet that should be used in future studies.

In summary, the results of this experiment show that genistein exerts its effects on the thyroid and reproductive hormone pathways differently in healthy individuals than in iodine deficient conditions, although the reproductive results were not significant. We can also infer that ample iodine intake should counteract any anti-thryoidic effects genistein may have.

APPENDIX

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

Provide information after each bold item
Student Name: Lindsey Nicole Hill

Student Email Address: Lindsey.hill@maryvillecollege.edu

Date: March 30, 2007

Senior Study Advisor: Dr. Drew Crain

Species to be used: *Xenopus laevis*

Age of animals: Stage 56 - metamorphosis

Number of animals in study: 30

Duration of study: August 2007-September 2007

Location of animals during the study (building and room) : 114 Sutton Science

List personnel to call if problems with animals develop:

Name	Daytime Phone	Nighttime Phone	Emergency No.
Lindsey Hill	256-0754		
Dr. Crain	981-8238	379-1706	
Dr. Swann	981-8068	981-5439	

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.

Submerge organisms in 0.05% MS222 solution

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

Maryville College IACUC Approval Number: ____________
Date Approved: ________________
Signed:
____

WORKS CITED

American Soybean Association. (2007). Soybean exports. *SoyStats, 7,* [Online Edition] <http://www.soygrowers.com/publications/soystats.htm>.

Bisschop, P., Toorians, A.W., Endert, E., Wiersinga, W.M., Gooren, L.J., & Fliers, E. (2006). Affects of sex-steroid administration on the pituitary-thyroid axis in transsexuals. *Eur J Endocrino., 155,* 11-17.

Bottner, M., Christoffel, J., Rimoldi, G., & Wuttke, W. (2006). Effects of long-term treatment with resveratol and subcutaneous and oral estradiol administration on the pituitary-thyroid axis. *Exp Clin Endocrinol Diabetes, 114,* 82-90.

Ceresini, G., Morganti, S., Graiani, V., Saccani, M., Milli, B., Usberti, E., Valenti, G., Ceda, G.P., & Corcione, L. (2006). Estrogen receptor (ER)-beta, but not ER-alpha, is present in thyroid vessels. *Thyroid, 16,* 1215-20.

Crain, D. A., Eriksen, M., Iguchi, T., Jobling, S., Laufer, G., LeBlanc, G.A., & Guillette, L.J. (2007). An ecological assessment of bisphenol-A: Evidence from comparative biology. *Reprod Toxicol, 24*, 225-39.

Divi, R.L., Chang, H.C., & Doerge, D.R. (1997). Anti-thyroid isoflavones from soybean: isolation, characterization, and mechanisms of action. *Biochem. Pharmacol., 54,* 1087-96.

Doerge, D.R., & Sheehan, D.M. (2002). Goitrogenic and estrogenic activity of soy isoflavones. *Environ. Health Perspect. 110*: 349-53.

Ji, L., Domanski, D., Skirrow, R.C., & Helbing, C.C. (2007). Genistein prevents thyroid hormone-dependent tail regression of *Rana catesbeiana* tadpoles by targeting protein kinase C and thyroid hormone receptor alpha. *Devel, Dynam., 236,* 777-790.

Kawahara, A., Baker, B.S., and Tata, J.R. (1991). Developmental and regional expression of thyroid hormone receptor genes during Xenopus metamorphosis. *Development, 112,* 4933-4943.

Kurahashi, N., Iwasaki, M., Sasazuki, S., Otani, T., Inoue, M., & Tsugane, S. (2007). Soy product and isoflavone consumption in relation to prostate cancer in Japanese men. *Cancer Epidemiol. Biomark. Prev., 16*, 538-45.

Lima, L.P., Barros, I.A., Lisboa, P.C., Araujo, R.L., Silva, A.C., Rosenthal, D., Ferreira, A.C., & Carvalho, D.P. (2006). Estrogen effects on thyroid iodide uptake and thyroperoxidase activity in normal and ovariectomized rats. *Steroids, 71,* 653-659.

Messina, M. & Redmond, G. (2006). Effects of soy protein and soybean isoflavones on thyroid function in healthy adults and hypothyroid patients: A review of the relevant literature. *Thyroid, 16,* 249-58.

Milerova, J., Gerovska, J., Zamrazil, V., Bilek, R., Lapcik, O., & Hampl, R. (2006). Actual levels of soy phytoestrogens in children correlate with the thyroid laboratory parameters. *Clin. Chem. Lab. Med. 44,* 171-4.

Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N., Hatyay, Y., Shimatsu, A., Kuzuya, H., & Nakao, K. (2002). Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J. Clin. Endocrinol. Metab. 87,* 5185-90.

Nieuwkoop, P.D., & Faber, J. (1994). *Normal Table of Xenopus laevis.* New York, NY: Garland Publishing, Inc.

Penza, M., Montani, C., Romani, A., Vignolini, P., Ciana, P., Maggi, A., Pampaloni, B., Caimi, L., & Di Lorenzo, D. (2007). Genistein accumulates in body depots and is mobilized during fasting, reaching estrogenic levels in serum that counter the hormonal actions of estradiol and organochlorines. *Toxicol. Sci.*

Poppe, K., Glinoer, D., Van Steirteghem, A., Tournaye, H., Devroey, P., Schiettecatte, J., & Velkeniers, B. (2002). Thyroid dysfunction and autoimmunity in infertile women. *Thyroid, 12,* 997-1001.

Poppe, K., Velkeniers, B., & Glinoer, D. (2007). Thyroid disease and female reproduction. *Cli. Endocrinol, 66*, 309-321.

Radovic, B., Mentrup, B., & Kohrle, J. (2006). Genistein and other soya isoflavones are potent ligands for transthyretin in serum and cerebrospinal fluid. *Br. J. Nutr. 95,* 1171-1176.

Rae, M., Gubbay, O., Kostogiannou, A., Price, D., Critchley, H.O., & Hillier, S.G. (2007). Thyroid hormone signaling in human ovarian surface epithelial cells. *J Clin Endocrinol Metab, 92,* 322-327.

Ryokkynen, A., Kukkonen, J.V., & Nieminen, P. (2005). Effects of dietary Genistein on mouse reproduction, postnatal development and weight-regulation. *Anim. Reprod. Sci. 93,* 337-48.

Szkudelska, K., & Nogowski, L. (2007). Genistein—A dietary compound inducing hormonal and metabolic changes. *J. Steriod Biochem. Mol. Biol, 105,* 37-45.

Taguchi, H., Chen, H., Yano, R., & Shoumura, S. (2006). Comparative effects of milk and soymilk on bone loss in adult ovariectomized osteoporosis rat. *Okajimas Folia Anat. Jpn. 83,* 53-59.

Walker, H.K., Hall, D.W., & Hurst, J.W. (1990). *Clinical methods: The history, physical, and laboratory examinations.* New York: Buttersworth.

Wantanabe, S., Yamaguchi, M., Sobue, T., Takahashi, T., Miura, T., Arai, Y., Mazur, W., Wahala, K., & Adlercreutz, H. (1998). Pharmacokinetics of soybean isoflavones in plasma, urine, feces of men after ingestion of 60 g backed soybean powder (kinako). *J. Nutr. 128,* 1710-15.

White, H.L, Freeman, L.M, Mahony, O., Graham, P.A., Hao, Q., & Court, M.H. (2004). Effect of dietary soy on serum thyroid hormone concentrations in healthy adult cats. *Am. J. Vet. Res., 65,* 586-91.