Effects of Thyroid Hormones on Embryo Development of the Zebra Finch (*Taeniopygia guttata*)

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Abstract:

Thyroid hormones have critical functions in vertebrate development and growth. Understanding the effects thyroid hormones have on development can lead to further understanding of how maternal deposition of hormones can effect embryos as a mechanism for adaptations. This experiment looks to see if thyroid hormone (T4 and T3) affects zebra finch (*Taeniopygia guttata*) embryo metabolism in the egg. The eggs oxygen consumption and carbon dioxide production was monitored at various intervals of development as a means to measure metabolism and development then were sexed using molecular methods. The data in this experiment indicate that low dosages of exogenous T3 and T4 may increase carbon dioxide production in the eggs while high dosages of T3 and T4 and the combination of T3 and T4 may decreased it. The addition of thyroid hormones increased the percentage of males developing in treated eggs. There was no evidence for sex specific effects of thyroid hormones on embryo metabolism. This research needs to be further analyzed.

Introduction:

Thyroid hormones are known for their affects on metabolism and development of vertebrates. As in mammals, thyroid hormone regulates body weight, fertility, and metabolism in birds (Merryman and Buckles, 1998). Thyroid hormones are synthesized in the thyroid gland. There are several forms of thyroid hormones, including thyroxine (T4) and triiodothyronine (T3) (Moyes and Shulte, 2008). Triiodothryonine (T3) is presumed to be the active form of the hormone and T4 is the hormone that is produced in the thyroid gland and then converted to T3 in target tissues. The hormones are hydrophobic making the process of diffusion out of a cell and the travel throughout the bloodstream easy (Moyes and Shulte 2008). These types of hormones

need a protein carrier in order to be transported in the blood since they cannot be dissolved in blood plasma, but can readily diffuse into the target cell because they can easily pass through the cell membrane, a trait generally in developmental hormones that need to travel throughout the body. Thyroid hormones are unique in that they tend to behave more like steroid hormones rather than peptide hormones although they are derived from a protein (Moyes and Shulte 2008). Comparisons are often made between thyroid and steroid hormones, a number of studies have evaluated the effects of steroid hormones on development, so knowing that thyroid hormones behave like steroid hormones allows these studies to be comparable.

Thyroid hormone function in avian species is fairly similar to that in mammals. In birds, thyroid hormones influence metabolic functions, development, growth, and reproductive functions (Merryman and Buckles 1998). There is a strong catabolic effect on the cardiovascular system in most mammals. Thyroid hormones play a role on the pattern of myosin isoform expression (Moyes and Shulte 2008). Thyroid hormones not only increase heart rate and myocardial contractility, they also increase myocardial oxygen consumption (Merryman and Buckles 1998). It is unknown if there is the same function in avian species therefore making the measurements of metabolic carbon dioxide production and oxygen consumption topics to be studied when looking at the effects of thyroid hormone in birds.

Thyroid hormones play an important role in development and differentiation of cells at development. Despite the essential functions of thyroid hormones in development, thyroid hormone production starts relatively late in vertebrate development (McNabb, 1997) and in mammals maternal thyroid status affects development suggesting that the embryo/fetus relies on maternal thyroid hormone production (Escobar-Morreale, 1995). In birds the development of the embryo appears to rely on maternal thyroid hormones that are deposited in the egg before it is

laid. It has been shown that thyroid hormones of maternal origin are present in variable concentrations in freshly laid chicken and quail eggs (Wilson and McNabb, 1997; Schwabl and Lindsay, unpublished). One study looked at thyroid concentrations in chicken eggs before and after the start of embryonic thyroid function (Prati, et al. 1992). The study found that thyroid hormones were available to the embryo throughout development even before the onset of thyroid function, leading to the idea that thyroid hormones are passed by the mother into the egg to influence embryo development.

Many of the hormones of a mother get passed to a developing embryo. Steroid hormones are one of the many hormonal signals an embryo receives from the mother. Considerable and variable amounts of androgens are deposited in the egg by the mother and these androgens, in particular testosterone affect many functions of the embryo (Groothuis et al, 2005). Hormone production is one of the major responses to a changing environment. A changing environment may result in maternal effects on offspring that modify their development and other traits (Groothius and Schwabl, 2007). This requires the need for understanding the changes in development due to specific hormones, such as thyroid hormones, that are very important metabolic hormones and influence many aspects of vertebrate development.

In developing sex characteristics avian species rely on Z-linked genes. Much like the mammals XX and XY chromosomes, avian species have a male ZZ and female ZW chromosomal organization (Chue and Smith, 2011). Although sex determination is mostly dependent on these specific genes, hormones can play a role (Paster, 1991). In one study, mother's treated with estradiol, a sex hormone, had a sex ratio with more females than males (Engelhardt, et. al., 2004). Maternal amounts of androgens deposited in the embryos have also been shown to influence the sex ratios to bias towards male (Adkins-Regan, et. al., 2013).

Although it is known that androgens can influence sex determination of embryos, there is no research determining the effects that thyroid hormones can play on the sex ratios.

In my study I tested the hypothesis that maternal thyroid hormones in the egg influence the embryo development. My experiment used injections of varying dosages of thyroxine, triiodothyronine, and control solvent.

Experimental Design:

In my experiment I used the altricial zebra finch (*Taeniopygia guttata*) as a model. This bird was a good organism to use for this study because the species breeds year round in captivity (Haddon, 1987). Moreover, the zebra finch is an altricial bird in which thyroid hormones appear to be produced only late in embryo development and after hatching (McNabb 1997). The usual clutch size for a zebra finch is around 5 eggs, and the embryos develop quickly with an incubation (embryo) period of 12 days (Haddon, 1987). Zebra finches are inhabitants of Australia and have been domesticated. I used domesticated zebra finches that have been bred in the lab since 2008.

The birds were kept in a controlled setting of a constant light/dark cycle (14L10) and ambient temperature (23C). They were monitored daily for any ailments, and fed an appropriate diet for birds their size and species. They were kept in cages of pairs of 1 male and 1 female and each pair were given a wicker nest to encourage mating and egg laying.

The eggs were collected from the birds by allowing the birds to lay them freely and retrieving them from their cages. The eggs were then injected with hormone solution or solvent and artificially incubated. The eggs were divided into five groups; control, high T4, low T4, high T3, low T3, and a combination of T4 and T3. I used a total of 85 eggs in this experiment. There were a total of 29 controls, 11 T4 high, 12 T4 low, 13 T3 high, 12 T3 low and 8 T4 and T3

combination. All of the eggs were injected using a 27g needle. The egg mass was recorded before injection. After injection the hole in the shell was sealed with a piece of flexible wound dressing (OpSite). The eggs were then placed in an incubator maintained at constant 37.5°C and 50-60% relative humidity (settings recommended by incubator company). At the fourth, eighth, and twelfth day of incubation I measured metabolism using Sable Systems respirometer. On the twelfth day after the last metabolism, the eggs were frozen in airtight containers for later analysis of embryo sex.

The hormones were dissolved in 10 ml of distilled water. The T4 high dose was 200 µL stock solution in 5 mL distilled water. The T4 low dose was 40 µL stock solution in 5 mL distilled water. The T3 high dose was 20 µL stock solution in 5 mL distilled water. The T3 low dose was 2 µL stock solution in 5mL distilled water. The combination of T4 and T3 dosages was 80 µL T4 stock solution and 8 µL T3 stock solution in 5 mL distilled water. The control group was injected with distilled water to control for the technique of injection. All of the eggs were injected with 5 microliters of each treatment and covered with a permeable tape to prevent leakage through the hole in the shell created by the needle. It was assumed that a zebra finch yolk was 300mg with a total T4 in the yolk was 18ng and total T3 in the yolk was 1.8ng. These values were assumed using the known values of T3 and T4 in quail yolk. Quails have an estimated 60pg/mg of T4 in yolk, and 6pg/mg of T3 yolk (Wilson and McNabb 1997).

After measurement of metabolism, embryos were collected from the eggs for determination of their genetic sex. The DNA was extracted from tissue using the Qiagen DNA extraction protocol. The methods for molecular sexing were derived from the Journal of Avian Biology (Fridolfsson and Ellegren, 1999). The DNA underwent a specified PCR cycle and was viewed using agarose gel electrophoresis.

Results:

At this time, the sample sizes are not large enough for proper statistical analyses. Results of the effects of thyroid hormone on metabolism are reported only for day 8 of incubation (days 4 and 12 will need to be analyzed). In the control group 41.3% of eggs showed a measurable response (CO2 production and O2 consumption); in the low T4 group 16.7% showed a response, and in the high T4 group 54.5% showed a response. The T3 low dosage group had 33.3% response, the high T3 had 50% response, and the T4 and T3 combination group had 25% response.

The data analysis through the program Sable Systems showed that the low dose treatments of both T3 and T4 tends to increase carbon dioxide production and oxygen consumption (Figure 1). The low sample sizes prevented statistical analyses of the results. The control average was 0.006% production and 0.003% oxygen consumption. The T3 low dose average was 0.01% carbon dioxide production and 0.006% oxygen consumption and the T4 low dose average was 0.007% carbon dioxide production and 0.01% oxygen consumption. The high dose treatments of T3 and T4 showed a decrease in carbon dioxide production when compared to the control (Figure 1). The T3 high dose average was 0.004% carbon dioxide production, no report for oxygen consumption, and the T4 high dose average was 0.003% carbon dioxide production and 0.002% oxygen consumption. The combination had a strong decrease in carbon dioxide production compared to the other dosages (Figure 1). The combination treatment had an average of 0.002% carbon dioxide production and no report on consumption.

The data revealed a sex ratio of the control embryos (n=6) of 80:20 (male to female). The T4 high dose (n = 4) had a 50:50 ratio while the T4 low dose (n = 4) had a 20:80 ratio (Figure 2). The T3 high dose (n = 4) and the T3 low dose (n = 4) each had a ratio of 70:30 (Figure 2). The

combination treatment did not have embryos that were developed enough to test for their genetic sex.

Sex did not have an apparent effect on embryo metabolism (carbon dioxide production) although sample sizes were too small for a statistical analysis (Fig. 3).

Discussion:

The incubation period for zebra finch embryos is twelve days (Haddon, 1987), however none of the eggs came to hatching, and were either dead or dying on the twelfth day. Day four of incubation is generally the stage in development where vasculature and heart begin to form (Haddon, 1987), making it difficult to obtain solid data at this stage. Day eight showed the most responses because there was good development in the heart and vasculature, and the eggs had good viability. For analysis on the DNA there was a lack of eggs with developed enough embryos to test their tissues for sex genes in their DNA.

The results showed that there was a difference between each of the treatments and that the hormones and their dosages did effect development in some way. The low dosages of each treatment had an increase in carbon dioxide production that could translate to an increase in development rate; this could be due to the fact that thyroid hormones are developmental hormones. However the effects of the high dose of each treatment was lower metabolism compared to controls. This could mean that the high dosages were pharmacological and was causing defects in embryo development. This data is certainly not concrete enough to draw any solid conclusions, however these preliminary results show that there is some effect of the addition to thyroid hormone on embryo development that might depend on dosage.

The results may also suggest that thyroid hormones could influence secondary sex ratio by differential effects on the sexes. According to the data, the addition of thyroid hormones

increased the percentage of males, however there is not enough evidence to draw a definite conclusion of the effects thyroid hormones play on sex determination. My results do not provide any evidence that sexes differ in embryonic metabolism.

The main factor hampering this study was a lack of adequate sample sizes. The overall embryo mortality was very high which could have been due to the incubator temperature and humidity conditions. More research is needed to identify the appropriate incubation conditions for zebra finch eggs. The eggs production rate of the birds was another factor reducing sample sizes for this study. The zebra finches used were of an advanced age reducing overall productivity.

Although this experiment needs further analysis, this study can and already is leading to further inquiries into the effect of thyroid hormone on temperate bird species.

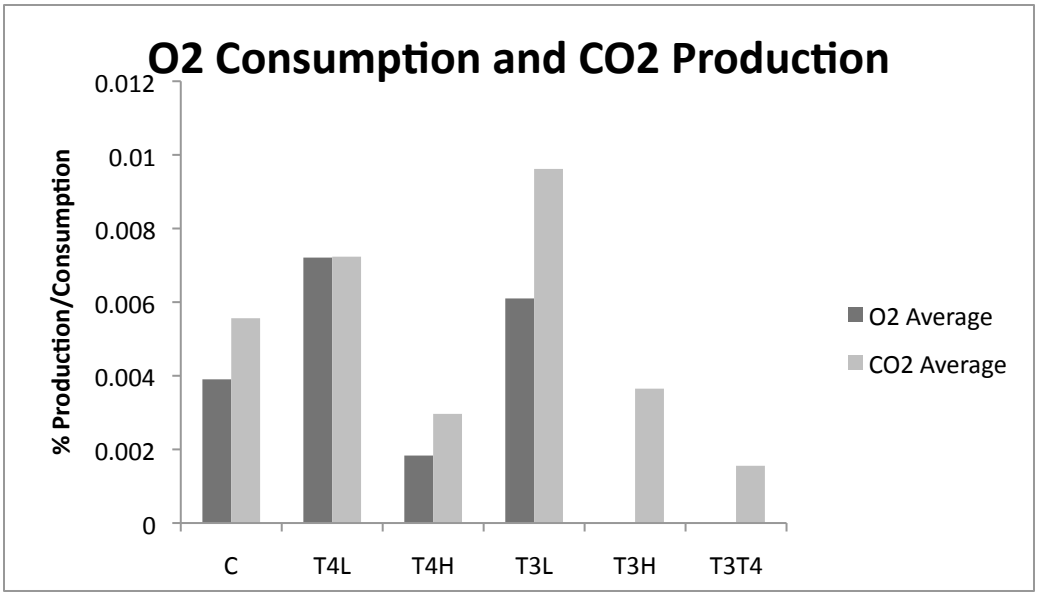

Fig. 1: Carbon dioxide production and oxygen consumption of zebra finch embryos treated with control solution (C), low and high doses of T3 and T4, a combination of low doses of T3 and T4. The measurements were performed on day 8 of egg incubation (i.e. 8-day old embryos). There were a total of 29 controls, 11 T4 high, 12 T4 low, 13 T3 high, 12 T3 low and 8 T4 and T3 combination.

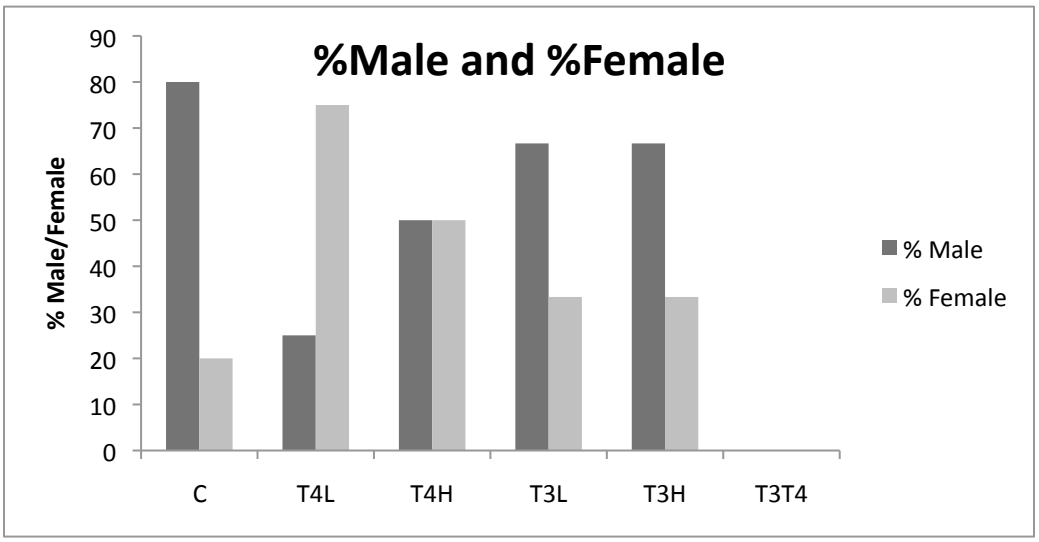

Fig. 2: The percentage of males and females within each treatment group. The control group only had six embryos that were tested. The other treatments had four embryos each tested. The sex was determined by using PCR and electrophoresis to reveal the ZZ or ZW bands.

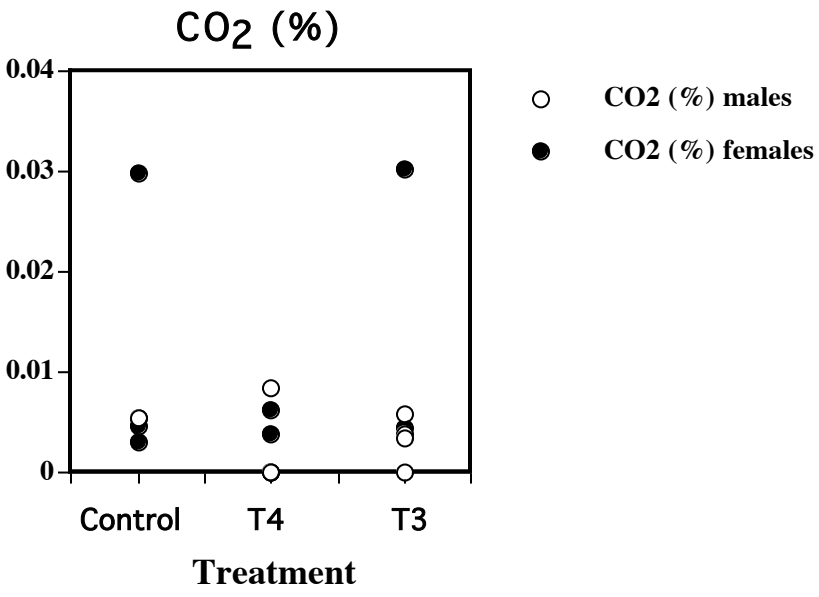

Fig. 3: Percent of carbon dioxide production of each treatment in comparison to sex of each treatment. The control treatment had six embryos tested for sex and the high and low treatments were combined in this graph giving a total of eight T4 and eight T3 embryos. Open dots are male and closed are female.

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