Plasma T_3 (Triiodothyronine) and T_4 (Thyroxine) hormone levels as reference values in rehabilitated healthy gray herons (*Ardea cinerea*)

Research Article

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ABSTRACT

In the rehabilitation of wild birds, determining the release criteria is very important for the survival of the animal after its release to the nature. When determining these criteria, the clinical examinations and clinical data of the animals should be evaluated. Therefore, reference laboratory values play a vital role in determining release to nature. In this study, 15 (fifteen) adults and healthy Gray heron (*Ardea cinerea*) were brought to the 'Kafkas University, Wild Animal Protection, Rescue, Rehabilitation Application and Research Center' clinic in May 2016, because their nests were destroyed as a result of the overturning of the plane tree in Iğdır province, and they did not leave their surroundings, were rehabilitated. Birds were kept in bird care rooms specially designed for their species in the rehabilitation center. The birds were taken blood 4 days after being rehabilitated and then released into the nature. Plasma triiodothyronine (T_3) and thyroxine (T_4) hormone levels in birds were determined as a result of the analysis of blood samples. It was concluded that the differences in plasma T_3 and T_4 hormone levels obtained from a total of 15 birds were due to the increasing physiological and metabolic needs during the captivity period in center.

Keywords: Bird physiology, captivity, Gray Heron, rehabilitation, thyroid hormones

INTRODUCTION

Thyroid hormones; thyroxine (T_4) and triiodothyronine (T_3) are critical for neurodevelopment, development of somatic cells and gonads in young animals (fetus, newborn and juvenile) (Nakao et al., 2008; Sørmo et al., 2005). They also play a role in regulating metabolism, thermoregulation, reproduction, and maintaining physiological homeostasis (Cherel et al., 2004; Cooke et al., 2004; Davis et al., 2000; Decuypere et al., 2005; Hudelson and Hudelson, 2009; Scanes and McNabb, 2003). Recently, studies have been conducted to investigate the relationship between thyroid hormones and many issues such as environmental factors (such as temperature, ligth, heat, humidity), keeping animals in captivity, the effect of contaminants (Baos et al., 2006; Cherel et al., 2004; Decuypere et al., 2005; Schmidt and Reavill, 2008). Environmental changes in wild birds cause significant physiological changes (Scanes and McNabb, 2003; Walker et al., 2005). As a result of these changes, the animal uses its available resources mostly for survival. And this puts extra physiological burden on the animal (Bennett et al., 2011; Davis et al., 2000). Especially keeping them in unsuitable cages or rooms may cause increased metabolism and stress in birds especially in rehabilitation centers (Bennett et al., 2011; Everds et al., 2013).

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In addition, basal metabolism and thyroid hormone metabolism may be affected in these centers by reasons such as odor, noise, light conditions, daily diet (Everds et al., 2013; Morgan and Tromborg, 2007). One of the aims of conservation biology is to understand the mechanisms of adaptation of wild animals to environmental conditions, what strategies they use, and the differences between individuals (Angelier et al., 2016).

In the rehabilitation of wild birds, it is important to determine when the bird will be considered healthy for release (Bennett et al., 2011). Therefore, in addition to clinical examination, the birds should also be evaluated hematologically, biochemically and physiologically (Guerra et al., 2018; Totzke et al. 1999). However, data on hormonal components in wild birds are very limited (Everds et al., 2013; Spagnolo et al., 2006). At the same time, since there are reference differences between species, intra-species comparisons can be one of the reliable parameters when assessing the health status of birds before release (Decuypere et al., 2005; Scanes and McNabb, 2003). Increased thyroid hormone levels in wild birds are thought to be more related to metabolic expenditures. In a study they conducted with *Rissa tridactyla* and *Uria lomviaher*, they investigated the relationship between basal metabolic rate, T_3 and T_4, and found that T_3 increased with basal metabolic rate in both species (Elliot et al., 2013). Chastel et al. (2003) similarly, in a study they conducted with *Passer domesticus*, found a directly proportional relationship between basal metabolism and T_3 hormone. Davis et al. (2000) in a study conducted with hens (Single Comb White Leghorn) kept in long-term cages, it was found that corticosteroid, T_3 and T_4 hormones change depending on egg production, and the hormone levels of hens with the highest egg production are also high.

In this study, no trauma or health problems were found in the physical examinations of Gray herons brought to the rehabilitation center. In this study, it was tested whether the practices during rehabilitation stressed the Gray herons and their relationship with T_3 and T_4 metabolism. After the Gray herons were rehabilitated, it was aimed to contribute to the literature by determining the plasma levels of T_3 and T_4 hormones just before they were released into the nature. In addition, understanding the physiology, hormonal status and thyroid metabolism of wild birds may help us determine conservation strategies of wild birds in the future.

MATERIAL and METHOD

In this study, 15 (fifteen) adults and healthy Gray heron (*Ardea cinerea*) who were brought to the 'Kafkas University, Wild Animal Protection, Rescue, Rehabilitation Application and Research Center' clinic in May 2016, because their nests were destroyed as a result of the overturning of the plane tree in Iğdır province, and they did not leave their surroundings, were evaluated. The aim of the rehabilitation of wild birds is to treat animals and release them back to the nature. Fort this reason, the international standards followed by the rehabilitation centers have been complied with in order to prevent any different reasons that could stress the birds during their stay at the center. In this process, considering that the procedure they experience for blood samples may cause stress, these birds living in captivity were contacted with the same physicians for minimum stress conditions. There was no unnecessary physical contact during the process and it was manipulated by the same medical staff during the blood collection.

No trauma or health problems were found in the clinical examinations of the Gray herons. They were rehabilitated in a center with optimal environmental conditions and species-specific shelter and flight areas (5 m/6 m/30 m). They were not disturbed for any reason other than the investigators' routine visit and were fed on white meat specific to the species. In order to prevent the birds from getting more stressed, no unnecessary medical intervention was made in the rehabilitation process. No blood samples were taken from these birds for hormonal examinations upon their first admission to the center. Therefore, there is no control group data showing the initial hormonal status of the birds in the study. Blood samples were taken 4 days after the birds were taken into the rehabilitation process, and all individuals were released back to the wildlife in suitable areas.

Collection of blood samples and analysis

As soon as the birds were brought to the center, they were taken to rehabilitation. After 4 days of rehabilitation, blood samples taken from ulnar / basilic venae and transferred to EDTA (BD vacutainer, K_2 EDTA) tubes were centrifuged at 3000 rpm (Electro-mag M815M) for 5 minutes and their serum was obtained. Serum samples obtained were stored at -20 ºC (Profilo 6600) until analysis. Chicken T_4 (Thyroxine, MyBiosource) ELISA kit and Chicken T_3 (Triiodothyronine, MyBiosource) ELISA kit was used to analyze the T_3 and T_4 hormones of the samples. The analyses were carried out according to the Elisa kit booklet.

Statistical analyses of data

The data were analyzed with SPSS 22.0 (Statistical Package for Social Sciences). The normality test of the data was performed using the Shapiro-Wilk test, and it was found that the groups were not normally distributed separately ($p<0.05$). Statistical analysis of blood samples taken from rehabilitated birds was performed by Chi-Square test and $p \leq 0.05$ was considered as significant.

RESULTS

In this study, plasma T_3 hormones were found to be statistically different in only 2 individuals and T_4 hormones in 6 individuals compared to the others (Figure 1 and 2).

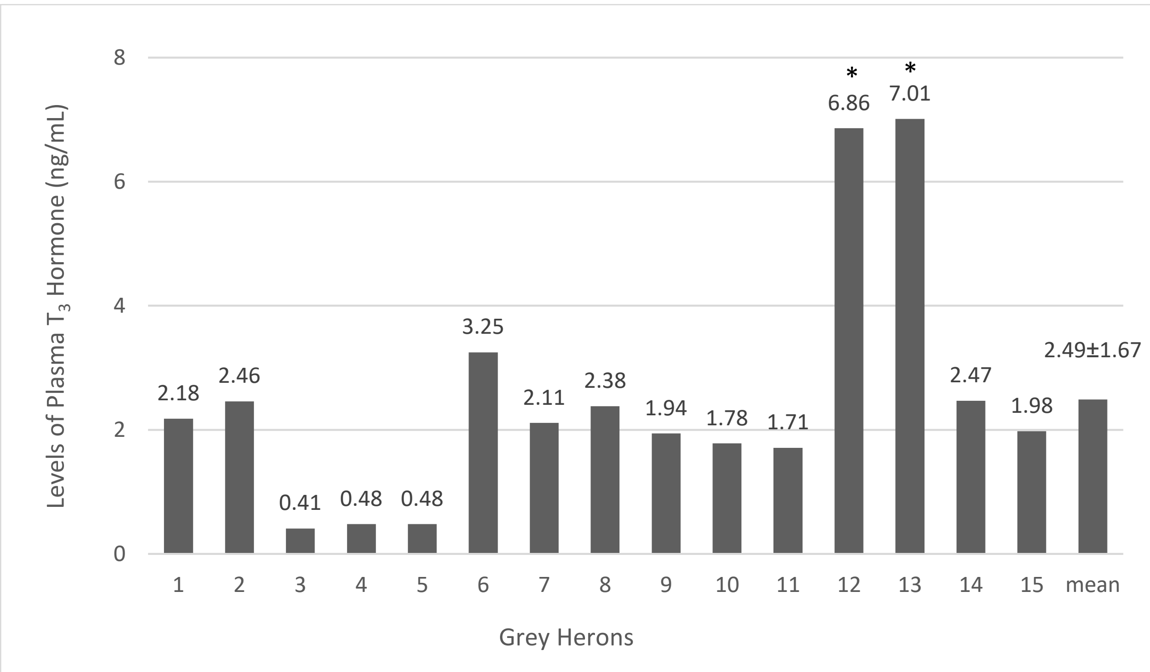

Figure 1. Plasma T_3 hormone levels of rehabilitated healthy Gray herons. *Statistically different individuals. Mean: Fifteen Gray herons' average plasma T_3 level (Mean ± Standart Deviation: 2.49±1.67). p= 0.011. Chi-square: 27.267.

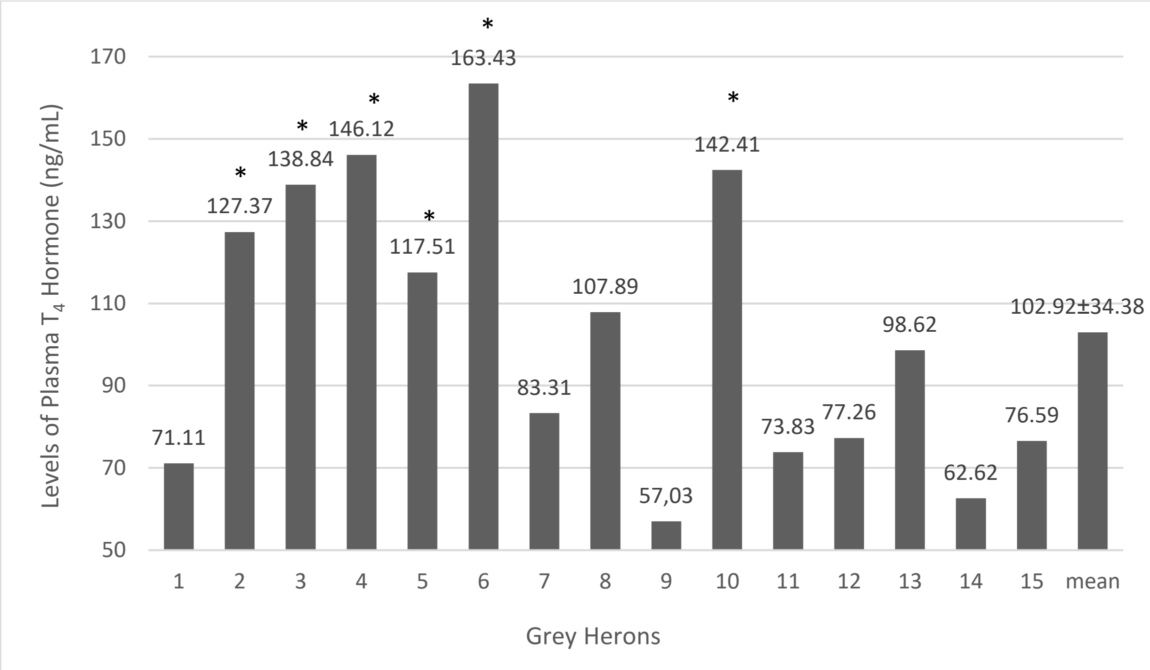

Figure 2. Plasma T_4 hormone levels of rehabilitated healthy Gray herons. *Statistically different individuals. Mean: Fifteen Gray herons' average plasma T_4 level (Mean ± Standart Deviation: 102.92±34.38). $p< 0.001$. Chi-square: 159.724.

DISCUSSION

The secretion of thyroid hormones is regulated by the hypothalamic-pituitary-thyroid (HPT) axis (Decuypere et al., 2005, Everds et al., 2013; Helmreich et al., 2005). There are two active forms of thyroid hormone circulating in living things. T_3 is the biologically active form. Because it binds to thyroid hormone receptors with higher affinity than T_4. Peripheral tissues can convert T_4 to T_3 by the action of 5′-deiodinase (Harr, 2002; Hudelson and Hudelson, 2009; Kaneko, 1997). However, T_4's rT_3 (reverse T_3) metabolism is faster than its conversion to T_3. Therefore, low T_4 levels may be present in the serum of birds when increasing amounts of rT_3 are produced (Decuypere et al., 2005). In this study, there was no statistical difference in plasma T_3 hormones in 13 individuals except 2 individuals. The increase in plasma T_3 hormones in these 2 individuals seems to be related to the increase in energy expenditure with restlessness.

Stress has an inhibitory effect on thyroid function in birds. Plasma cortisol levels also increase with stress. Increased plasma cortisol level can lead to inhibition of thyroid hormone production or a decrease in deiodination of T_4 to T_3 (Chastain and Panciera, 1995; Palme et al., 2005). Therefore, high cortisol levels may result in decreased T_4 and increased T_3 in serum, or both to decrease (Baos et al., 2006). It has been reported that the increases in T_3 are due to the increase in energy expenditure caused by the stress of the animal (Angelier et al., 2016; Fischer and Romero, 2018; Helmreich et al., 2006; Wentworth et al., 1986; Zoeller et al., 2007). Groscolas and Leloup (1989) found that in captivity Emperor penguins (*Aptenodytes forster*) had three times lower plasma T_4 concentrations than free-living penguins. It is known that cortisol levels increase in birds in captivity or due to activities such as ringing, holding taking blood, and there are many studies on this (Walker et al., 2005). However, studies on the effects of thyroid hormones in birds kept in captivity are not enough yet. Bennett et al. (2012), in a study they conducted with seal pups (*Halichoerus grypus*), suggested that during the research, the researchers' movement in the colony for blood sampling and morphological measurements could only cause a general disturbance to the colony. In the present study, it is thought that the changes in plasma T_3 and T_4 hormones are caused by the increased metabolic needs of the birds, and the disturbance caused by the researchers for blood intake does not affect the levels of thyroid hormones in the birds. DeRango et al. (2019) in a study with fur seals (*Arctocephalus philippii townsendi*) found a positive relationship with T_4 and rT_3 in adult females and a negative relationship with T_3 in response to capture stress. In the present study, the increase of T_4 and lower T_3 suggest that it is due to increased metabolic needs and functions as a resource to be converted into rT_3.

Welcker et al. (2015) found a positive relationship between basal metabolic rate and T_3 in a study they conducted with *Rissa tridactyla*. They suggested that factors such as catching and holding the bird could temporarily change T_3 levels. In the present study, there was no difference in T_3 hormones except 2 healthy individuals. This difference may be due to temporarily increased basal metabolic rate changes in 2 individuals (Welcker et al., 2013; 2015).

CONCLUSION

At the end of the rehabilitation processes of the healthy Gray herons in this study, it was aimed to determine the levels of thyroid hormones, which were not previously reported in the literature. Depending on the procedures applied in the center, i reported in the study, i believe the data can be evaluated as thyroid hormone values of healthy individuals. In addition, the stress they experienced during the rehabilitation process was also taken into account. In addition, these data may not fully represent the expected values for free-flying Gray herons under natural conditions, due to the individual effects of feeding and captivity stress. Due to the low number of subjects in the study, these data i present can be evaluated as preliminary data for studies that can be carried out with a larger number of subjects.

In conclusion, I believe that plasma thyroid hormone values determined with healthy wild Gray herons in this study will contribute to the evaluation of similar studies to be conducted in the future, in the sense of “reference values” as the first reported data.

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