EFFECT OF TEMPERATURE STRESS ON THYROID FUNCTION IN NORMAL ADULT RATS

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(The Study Was Carried Out At Makerere University, College Of Health Sciences, Mulago)

1: *ABSTRACT*: *The thyroid gland is an important gland that secretes thyroid hormones such as T_3 and T_4 which control various life processes and metabolism in cells. Most of the available literature on thyroid gland function is limited to humans who sweat and less furred but knowledge on furred and non-sweating animals like rats which could serve as a good representative in this aspect is inadequate. Prior to the study, a confounding study was carried out on the effect of starvation in normal adult rats. A total of ten rats (Rattus norvegicus) were used, five were left to starve, and the rest were well fed on rat pencils bought from Nuvita feeds. The results revealed a significant ($p=0.001$) reduction in thyroid function in the starved rats. On this basis, therefore, throughout the study, all the rats were given the same treatment except in regard to feeding, oxygen concentration, light, genetic factor, and age except temperature treatment. The blood for testing the thyroid hormones was collected by cutting a small piece of the tip of the tail into an anticoagulant (heparin) bottle. The data collected were analyzed by the computer program XL--STAT 2008 version. One way-ANOVA was used to test for any significant increase or decrease in mean production ofT_3, T_4TSH, and FT_4andpost Hoc, Tukey test was used to locate the increase or decrease. The findings showed that for both hypothermia and hyperthermia registered increased and decreased levels of T_3 and T_4 respectively. Unlike FT_4levelwhere its level increased in hyperthermia and decreased in hypothermia, TSH, decreased in hypothermia and unaffected in the hyperthermia.*

2: INTRODUCTION

The thyroid gland is an endocrine gland that secretes triiodothyronine (T_3) and tetraiodothyronine (T_4) among other hormones, [1].Thyroid hormones play a pivotal role and are critical in the central regulation of body temperature (T_b), stimulation of thermogenesis, and regulation of cellular metabolism[2]. Other functions of the hormones include; normal cardiac functioning, growth, development, and reproduction.

The secretion of triiodothyronine (T_3) and thyroxin or tetraiodothyronine (T_4) are tightly and sensitively regulated by the negative feedback control system via the hypothalamus-pituitary gland axis [3]. This tightly regulated feedback control system may be affected by exogenous and endogenous factors[4]. These factors may profoundly also produce alteration in various aspects of the body functions which include; thyroid gland functions and metabolism just to mention but a few [5]. Any alteration in thyroid function would result in a cascade of metabolic effects related to its function. For example, body weakness and fatigues, difficulty in sleeping, increased sensitivity to heat and cold, weight loss and gain, dry irritated, puffy, and or bulging eyes, dry and puffy skin, hair loss, hand rumors, and increased heart rate to mention but a few [2]. All these put a risk on the survival and conservation of animals both domesticated and in the wild. This, in turn, would be devastating to the life of human both economically and nutritionally[6].

Most of the studies that have eventually ended up being extrapolated to represent human situations, trace their roots from lower mammals including rats and mice, and the reliability of the results obtained depends on the normal physiology. So, any factors (of which temperature could be one of them), that could affect the normal physiology of these models would affect their use as experimental animals and results obtained may be misleading and may not appropriately be extrapolated to represent human situations. This is especially useful most especially at the time, where the phenomenon of global warming is on increasing debt worldwide and would, therefore, result into abnormally high or low temperatures that could alter normal human physiology [7]. Therefore, the need to assess the effect of temperature stress on the thyroid gland functioning in furred and non-sweating mammals and rats as models is paramount in this regard. This study is therefore aimed at addressing the effect of temperature stress on the thyroid function. This was done by using indicators like the corresponding pituitary hormones (like Thyroid stimulating hormone(TSH)or thyrotropin-releasing hormone (TRH)thyroid gland and plasma hormones which could give information on how the rate of metabolism changes with environmental temperatures([6].

3.0 MATERIALS &METHODS

3.1. Type of Study

The study was a laboratory horizontal experimental study on an eight-month-old normal adult rat. They were housed in the same cages for at least one month to avoid light variations and their effects on thyroid function. Constant food and water were supplied to the animals, right from the time they were weaned and throughout the experimental period to avoid the effects of starvation and fasting on the thyroid function. The food was constituted by rat pencils bought from Nuvita Feeds and was assumed to contain a balanced diet.

Animals were kept in the Small-animal laboratory at the Physiology department of Makerere University, College of Health Sciences, and Mulago. Rats were kept at room temperatures at 25 ^{0}C, using a thermostat that switches on cooling fan or heater automatically to avoid the effect of cold or increased temperature on thyroid function of rats prior to the experiment.

3.2 Scope of the Study

The study was limited to a total of sixty (60) adult normal rats of species *Rattus norvegicus.* It specifically assessed the effect of temperature stress on the thyroid function basing on the thyroid hormones of triiodothyronine (T_3) and tetraiodothyronine (T_4) free tetraiodothyronine (FT_4) and corresponding pituitary indicators thyroid stimulating hormones (TSH). Rats in the study were subject to only two temperature extremes ie high and lower temperatures at 41 ^{0} C and 10 ^{0}C respectively with respect to the control at prevailing room temperature of 25 ^{0} C regulated by a thermostat. This study took a period six months from proposal development to article or manuscript production.

3.3.Experimental Procedure

A total of sixty rats were involved in this study, nine of which were from the same litter to minimize the effect of a genetic factor on the thyroid gland function. They were then divided into three treatment groups of equal numbers (20). In each group, three rats from the same litter were included in order to minimize the effect of genetic factor as shown in table 1. The animals in the first two groups, each consisting of twenty (20), were subjected to conditions of two temperature extremes for two hours i.e. they were put in the same well aerated chamber that had a thermostat set at 41 ^{0}C and10 ^{0}C, respectively with an incubator fixed to the respirator tube used to supply the necessary air. Rats in the third group that served as control were left in their cages at the prevailing room temperature of 25^{0}C maintained by a thermostat for the same duration.

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Table 1: Characteristics of the Study Population

Total population of the study	Hypothermia (10 ^{0}C)	Hyperthermia (41 ^{0}C)	Control (25 ^{0}C)	Body mass (g)	Diet	Genetic factor
60	20	20	20	28.87	Rat pencils	9 (total) 3 (each)

3.4: Measurement of Body Mass

The mass of each rat was taken and recorded by wrapping each rat in a black polythene bag and then weighed on the digital scale. The mass of the polythene bag was then deducted from the total mass and so the remaining mass was for the rat in question.

3.5: Collection of Blood

Blood for testing thyroid function indicators, i.e. TSH, T_3, and T_4 was collected by cutting a small piece at the tip of the tail. Blood was squeezed out of the tail into a collection bottle containing an anticoagulant (heparin) before samples were taken to the radioimmunoassay laboratory whereT_3, T_4 and TSH levels were measured as outlined below.

3.6: Experimental Procedures of Radioimmunoassay (RIA)

The RIA method is based on the competition between unlabelled TSH, T_3, and T_4 and a fixed quantity of ^{125}I labeled TSH, T_3, and T_4 for the limited number of unlimited sites on TSH, T_3 and T_4 for specific antibody. The samples of ^{125}I were incubated for 2 hours to allow them to react with a fixed antigen of tracer and antibody with different samples of unlabeled ligands. The amount of the tracer bound by the antibody was inversely proportional to the concentration of unlabeled ligand. During the two-hour incubation period with continuous agitation, immuno-complex was immobilized on the reactive surface of the test-tube. After incubation, the reaction mixture was discarded and radioactivity was measured in a gamma counter. The concentration of antigen was inversely proportional to the radioactivity of the reagent measured in test tubes. By plotting graphs of the binding values against series of calibrators containing a known amount of TSH, T_3, and T_4 calibration curves were constructed by the computer itself, from which the unknown concentration of TSH, T_3, and T_4 were determined in the blood sample.

3.7: Determination of the Concentration of TSH, T_3, and T_4 in Blood of Rats (*Rattus norvegicus)* **Subjected to Different Conditions**

The radioactive coat was put on by the researcher in order to be protected from health hazards of radioactive substances.

Test tubes coated with an anticoagulant (heparin)were purchased from Mulago hospital. They were then labeled in duplicate for each **standard** (S_1-S_5), **control serum © a**nd two test tubes for the total count (T). 10 µl each of the **samples (M)** was carefully pipetted using a micropipette into the labeled samples. At the same time300 µl of the tracer's reagent were pipetted into each of the labeled tubes. All the tubes were gently vortexed, sealed with plastic foil, and put in the test-tube racker which is firmly fixed onto the shaker plate. The shaker was turned on and adjusted to an appropriate speed such that the liquid rotated constantly. The tubes were incubated at room temperature for 2 hours. 2 ml of diluted wash buffer was added to each of the tubes and the supernatant from all the tubes was decanted by inversion of the rack in the upside position, the rack was placed on an absorbent paper for 2 minutes[8]. These steps were repeated and the concentration of the thyroid hormones was determined by using a gamma counter for 60 seconds. The average serum concentrations, the mean of three consecutive measurements were taken on different days using the same rat from calibration curves read from the machine by interpolation. This was automatically done by a computer itself with an incorporated computer software program meant for this purpose.

TSH, $T_3$and T_4 in each tube were calculated basing on the formulae given below, which are all incorporated in the RIA machine itself,

For the standard, B /T% =S_1 cpm/T *100 (optional).

The rest of the sample, it was calculated from B/B% = S_2-

S_5/C/M/S_1 (cpm)*100(cpm).
Where;
T= total count,
S_1= Zero standard,
S_2-S_5 = Standards/T%=% binding site per minute,
B/B%= % Normalized percent bindings,
cpm = Count per minute
C =Control, M= mean count).
Using semi-logarithmic graph paper B/B % for each standard, the corresponding concentration of T_3, T_4, TSH &Free thyroxin (FT_4) (from calibration curves) were constructed from which the unknown concentration of each hormone in the sample was determined by interpolation. All these were done by the computer itself incorporated with a computer program, XL-STAT 2009 meant for this purpose.

3.8: Data Analysis

Data were analyzed by use of computer program, **STATISTICS for XL-STAT 2009 VERSION.** A one way-ANOVA was used to test whether there was any significant increase or decrease in mean production of T_3, T_4, TSH, and FT_4 as influenced by coldness and heat on thyroid function. Tukey's test was further used to locate the increase or decrease of the levels of T_3, T_4 TSH and FT_4 as influenced by the coldness and heat.

4.0: RESULTS

4.1: Effects of Temperature Stress on T_3 Levels

The results showed that hyperthermia tended to depress the thyroid function and this resulted into decreased mean T_3 production as indicated by the very low mean of 107.45 ± 6.144 ng with respect to the control with a mean value of 127.130 ± 2.344 ng (Table 2). However, hypothermia slightly increased the mean T_3 production (Table 1). Tukey's test between the means T_3 production for the hypothermia and control, showed no significant increase (p=0.428), while a comparison between the mean T_3 production for hyperthermia and Control showed a significant increase (p=0.0001). Thus, while increased ambient temperatures decreased thyroid function, hypothermia increased it.

4.2: Effect of Temperature Stress on T_4

The finding showed that higher temperature decreased the mean production T_4 to a very low mean of (0.87 ± 0.043 µg/dl) which is an indicator of decreased thyroid function. While lower temperature increased mean T_4 production as seen from the highest mean of (4.819 ± 0.043 µg/dl) with respect to the control with a mean T_4 production of (4.31 ± 0.129 µg/dl) which is indicative of increased thyroid function. Tukey's test between the mean T_4 production for hypothermia and control showed a significant increase (p=0.0001). On the other hand, a comparison between mean T_4 production of hyperthermia and Control showed a significant decrease (P=0.0001). Hence, mean T_4 production is significantly influenced by both temperature extremes (Tables 2 and 3& figure 2).

4.3: Effect of Temperature Stress on TSH Mean Production

Hypothermia decreased the mean TSH production to a very low mean value of 0.033 ± 0.002 µiu/dl (Table 2 & figure 3) as compared to the control with a mean TSH production of 0.041 ± 0.003 µiu/dl. Hyperthermia does not affect TSH as the mean TSH production with respect to the control(Table 2). Tukey's test between the mean TSH production of hypothermia and control showed a significant decrease (p = 0.001). While a comparison between the mean TSH production of control and hyperthermia showed the insignificant change (p = 0.986). Hence, the thyroid function was significantly influenced by low-temperature extremes as depicted by a decreased level of TSH (Table 2).

4.4: Effect of Temperature Stress on FT_4 Mean Production

The result showed higher and lower temperatures increased and decreased FT_4, respectively as seen by the very high and low means FT_4 production 3.690 ± 0.136 µiu/ml and 0.622 ± 0.038 µiu/m for hyperthermia and hypothermia, respectively, as compared to the control (Table 2 & 3 and figure 4). Tukey's test of the difference between the mean FT_4 production of hypothermia and control showed a significant decrease (p = 0.0001), while a comparison between the mean FT_4 production for hyperthermia and control showed a significant increase (p=0.0001). Hence, FT_4 is significantly influenced by both temperature extremes (Figure 4 and Table &1& 2).

5.0 DISCUSSION

5.1: Effects of Temperature Stress on T_3 Levels

The findings indicated higher temperatures (hyperthermia) insignificantly (p=0.428), decreased T_3 levels in blood an indicative of decreased thyroid function with respect to the control. This was like the study conducted by Mancini, Festa, *et. al.* [9], whereby it was reported that T_3 levels in blood decreased with increased temperature in euthyroid patients. This is because biosynthesis of T_3 is strictly enzymatically controlled by thyroid peroxidases which are very sensitive to temperature deviations from the optimum and also its conversion to diiodothyronine (T_2). Therefore, an increase in temperature resulted in the denaturation of active sites and the devastating effect on the activity of the pituitary-thyroid axis function as depicted from low levels of T_3 in blood [9].

On the other hand, low temperatures (hypothermia) significantly (p=0.0001) increased T_3 blood levels in blood with respect to the control, an indication to increased thyroid function and hence metabolism. These finding differed from those reported by Sean *et al.*, [7] whereby a significant reduction in thyroid function in guinea pigs exposed to low temperatures, basing on the plasma iodide values, but their assessment did not base on the thyroid hormonal level in blood. This was because they used plasma iodide to measure thyroid function and iodine being a raw material would be expected to decrease since it was used as a raw material for the biosynthesis of thyroid hormones of which T_3 is one of them [10]. Since the thyroid hormones control metabolism, decreased temperature would result in increased metabolism which necessities more synthesis T_3 and thus, its release in the bloodstream. Even then, the experimental animal used was of different species and cannot overturn the findings reported in this study. For example, whereas their study concentrated on the guinea pigs and rodents, this study was based on normal adult rats. This is because the response to

thyroid functions on temperature stress varies from one species to another and even within species themselves depending on age, sex, body size among other. However, they concluded that as an adaptation to the cold, this was accompanied by an increase in the secretion of thyroid hormones.

5.2: Effect of Temperature Stress on T_4 Mean Production

Hyperthermia significantly (p =0.0001) decreased the thyroid T_4 blood with respect to the control. The decrease in T_4resulted into a reduction of T_3 blood level. This is because T_4 is considered a precursor for the formation T_3, the active form required to boost increased metabolism. Thus, elevated temperatures directly deterred the synthesis of T_4due to decreased metabolism. This is in agreement with studies conducted by Tao, Zhang, Dong, Zhang, & Xin, [12], who observed that the blood level of both T_4 and $T_3$of broilers was reduced significantly on exposure to the temperature stress. They, however, stressed that the differences in the T_3 concentration were more expressive, thus, the variation is more accurate in explaining its active role in metabolism. Thus, higher temperatures appear to have similar effects on the thyroid functions in normal adult endothermic animal irrespective of body size and species.

The results also indicated that T_4 blood level were significantly (p=0.0001) increased in rats on exposure to lower temperatures with respect to the control. This was because decreased temperature results into increased metabolic rates as abide to maintain constant body temperature in an endothermic animal including rats. The thyroid hormone plays vital roles in this perspective, T_4 is considered as a prohormone required for the synthesis of the active form of thyroid hormone, the T_3. Thus, decreased environmental temperature facilitates the production more T_4 which is eventually converted to T_3, active form to increase the required metabolism. Similar observations were made by (Decherf et al., 2010)[6] whereby the blood T_4 concentration in fowl, increased on exposure to hypothermia. They attributed this to the increased metabolic rate of which T_4 is a prohormone needed in the formation of T_3, an active hormone in embolic activities in the body. Thus, the response to hypothermia in endotherms does not differ irrespective of their distance in the evolutionary tree.

5.3: Effect of Temperature Stress on the TSH

The result indicated that hyperthermia (higher temperature does not affect the mean TSH production with respect to the control (p= 0.986). This is partly because TSH is secreted by the pituitary gland via the pituitary-thyroid axis in accordance with the demand for T_3 which plays an active role in metabolism. Thus, hyperthermia results in a reduction in the metabolic rates and accordingly the secretion of TSH which is a stimulating hormone in the secretion of T_3 decreases. Secondly increased temperature results into denaturation of the active site of the enzymes needed in the catalyzing of the biosynthesis of TSH in blood. Unlike the studies conducted by Silvestri, Schiavo, & Lombardi [11] whereby it was availed that the concentration of TSH in layer birds was reduced significantly on exposure to prolonged heat and the difference was attributed to variation in species used in their studies.

The findings indicated that hypothermia significantly (p=0.0001) decreased TSH mean production. This is because lower temperatures inactivated the enzymes which are responsible for the facilitation of biosynthesis of TSH and its release in blood, thus reduced levels in the blood. In line with findings reported by Nelson & Habibi [2], who found out that response to environmental stresses in most subterranean rodents exhibits low metabolic rates and relatively low thyroid gland activity and hormone secretion are elevated in response to prolonged cold exposure, conforming to mammalian norms. Thus, it appears that the nature, magnitude and duration of exposure to low temperatures in mammals vary considerably.

5.4: Effect of Temperature Stress on FT_4

The result indicated that both hyperthermia and hypothermia significantly (P=0.00001) increased and decreased FT_4 blood levels respectively. The increase of FT_4 during hyperthermia is due to increased metabolic rates, which result into the release of more FT_4 from the peripheral sources such as the kidney, liver, and lungs, in order to be converted to T_4 and finally T_3 to support the increased metabolism. Decherf *et al.*[6] and Gisele *et al.* [4] reported a decreased FT_4blood level in mammals during hypothermia. This is because they used humans in the experimental models and generalized to all other mammals, yet humans are hardly furred and also sweat unlike other mammals such as rats used in the current study. Additionally, other such factors like; age, light variation, nutrition among others, which affects the thyroid gland could not have been kept constant [9].

The higher physiological range of FT_4 shown in Table 2, in comparison with 0.89 ±0.045 ng/dl recorded by Edeline, Bardonnet, *et. al.* and Nelson, E. *et. al* [13,2] could be explained by dissimilarities in experimental animals and the spectrophotometer method of analysis was used in the determination of thyroid hormonal level. For example, they used guinea pigs and rabbit whose thyroid gland functions were reported to have significantly reduced exposure to low temperatures at 12^0C. They also used spectrophotometric method which is less sensitive, unlike this where radioimmunoassay used in this study [2].

6.0 CONCLUSIONS

From the findings in this study, the following conclusions can be drawn;

- Hyperthermia (higher temperatures) significantly decreased T_3 production while hypothermia (lower temperatures) insignificantly decreased it.
- Both higher temperatures (hyperthermia) and lower temperatures (hypothermia) significantly increased and decreased$T_4$and FT_4production.
- TSH production insignificantly decreased thyroid function under higher temperatures (hyperthermia) but significantly increased under lower temperature (hypothermia).
- Both temperature extremes had a significant increase and decrease on FT_4 production.

7.0 Areas for Future Research

The following areas would be recommended for further future research;

- A similar study may be conducted on other mammals other than man and rats living in Sub-Sahara Africa.
- A study on the effect of temperature stress on thyroid function of varying age groups of rats (vertical study) may be conducted.
- Other factors like light and nutrition known to affect thyroid function would be investigated on thyroid function in normal adult rats.

8.0: Declaration of Areas of Interest

I declare that there is no conflict of interests that could be perceived as prejudicing and impartiality of the research reported. This research is purely meant for academic purpose and has nothing to do with employment and consultancies, grant fees and honoraria, ownership of stock among others.

9.0: Funding

The current area of study was entirely funded by the researcher.

10.0: Authors' Contributions

Some work on the effect of temperature stress on the thyroid function in various mammals with man inclusive has been conducted. However, the available literature is only limited to normal animals' species other than normal adult rats. Even then the available literature that exists is on diseased rats living in the Mediterranean which are in cooler places than those in Sub- Sahara Africa which is relatively hotter. Thus, the findings reported by the previous researchers may be falsifying to the prevailing condition in Sub-Sahara Africa most especially when using rats as experimental models. Thus, the current study provided a basis on effect temperature stress on normal adult rats living Sub Sahara Africa as they cheaper and most readily and cheaper to be used as experimental models. The results indicated that rats under temperature stress should not be used as experimental models, because their physiology is altered and so the results obtained would be falsifying if extrapolated to represent human situations.

12.0: Tables of the Findings

Table 2: Effect of Hypothermia on the Thyroid Function

Thyroid hormone	Variables	Control	Hypothermia	p-value
T_3	Mean value	127.13	128.78	0.428
T_4	mean value	4.311	4.819	0.0001
TSH	mean value	0.041	0.033	0.0001
FT_4	mean value	1.147	0.622	0.0001

Table 3: Effect of Hyperthermia on the Thyroid Function

Thyroid hormone	Variables	Control	Hyperthermia	p-value
T_3	Mean value	127.13	107.45	0.0001
T_4	mean value	4.311	0.870	0.0001
TSH	mean value	0.041	3.696	0.986
FT_4	mean value	1.147	0.041	0.0001

13.0: Figures of the Findings

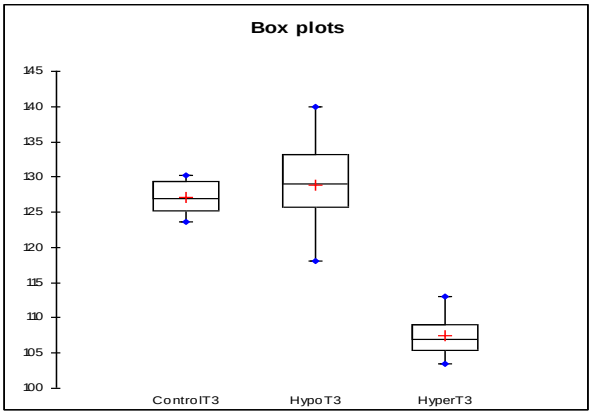

Figure 1: Effect of Temperature Stress on T_3 Mean Production.

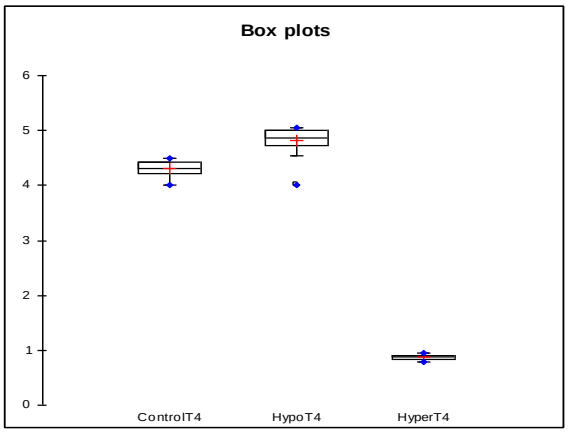

Figure 2: Effect of Temperature Stress on T_4 Mean Production

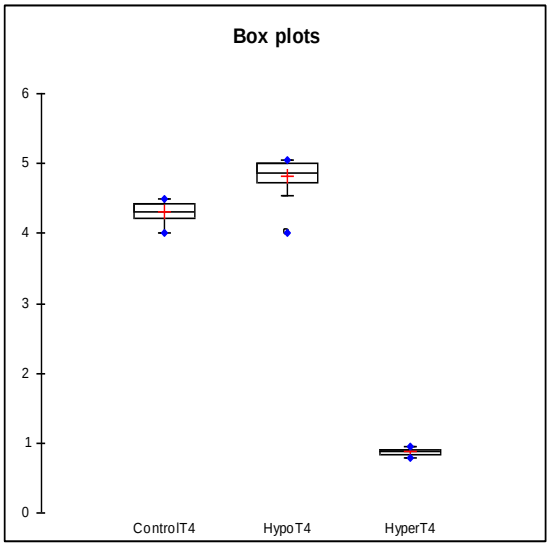

Figure 3: Effect of Temperature Stress on T_4 Mean Production

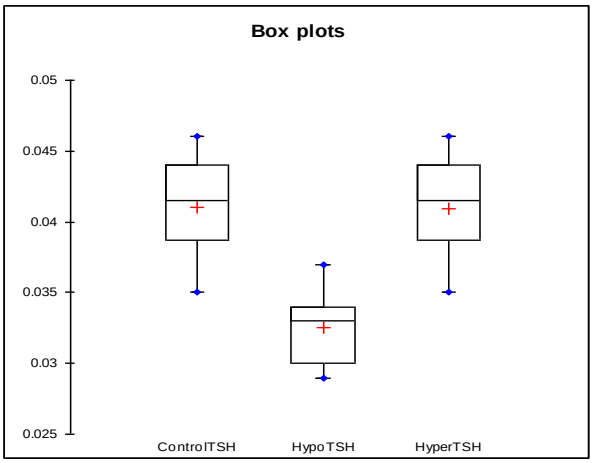

Figure 3: Effect of Temperature Stress on TSHMean Production

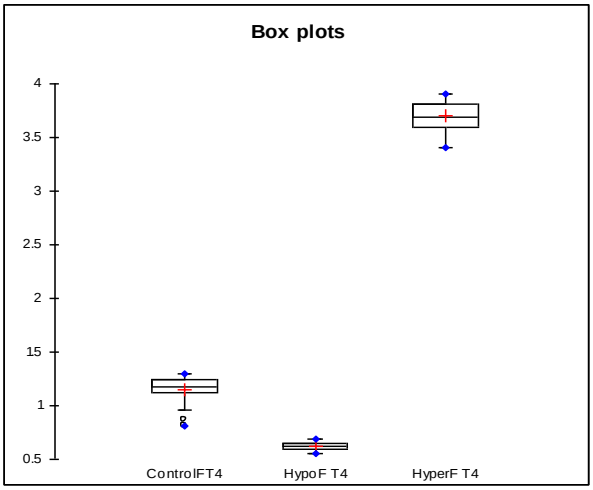

Figure 4: Effect of Temperature Stress on FT_4 Mean

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