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EXPERIMENTAL ANIMAL MODEL OF THYROID DYSFUNCTION - HYPERTHYROIDISM

Natasha Stojkovska[1], Nevena Manevska[2], Tanja Makazlieva[2], Ljubica Tasheva[1], Toni Tripunoski[2], Sinisa Stojanoski[2], Anamarija Paunkoska[3], Irena Kostadinova Petrova[1]
[1]Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Institute of Histology and Embryology, Skopje, North Macedonia
[2]Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Institute of Pathophysiology and Nuclear Medicine "Acad. Isak S. Tadzer", Skopje, North Macedonia
[3] Ss Cyril and Methodius University in Skopje, Faculty of Medicine, Institute of Anatomy, Skopje, North Macedonia

Abstract

To establish an experimental animal model of impaired function of the thyroid gland – hyperthyroidism. Thyroid dysfunction (hypothyroidism, hyperthyroidism) is classified in the group of non-communicable diseases with high incidence and prevalence. The disturbed function of the thyroid gland reflects in the metabolic processes of organs, tissues and at cellular level.

Twenty Wistar rats (10 males - 350 ± 40 gr and 10 females - 320 ± 40 gr), with an average age of 7 months, were analyzed in the period of 2 months (treated for 1 month with pharmacological doses of L-Thyroxin 15μg / 100g body mass in the drinking water and 1 following month without treatment). The second group of 10 Wistar rats - (5 males and 5 females) did not recieve L-Thyroxin and this was the control group used to obtain normal morphometric and biochemical parameters.

Between the mean values of FT4 and FT3 at the beginning, at the intersection and at the end of the study, there were statistically significant differences with increased levels of thyroid hormones at the cross section and no statistically significant difference of thyroid hormone levels between the beginning and the end of the examination in the group of rats treated with L-Thyroxin. No statistically significant differences were detected in the control group of rats. Experimental animal model of impaired function of the thyroid gland – hyperthyroidism using Wistar rats as subjects was successfully established.

Keywords: hyperthyroidism; thyroid dysfunction; thyroid hormones; Wistar rats;

Introduction

Thyroid hormones are known to have multiple effects on various tissues and organs. They regulate the basal metabolism, the metabolism of proteins, fats and carbohydrates and influence the growth and differentiation of almost all cells and tissues in the body. Of the total amount of secreted hormones, about 90% is thyroxine (T4), and only 10% is triiodothyronine (T3), but in the peripheral tissues, T4 converts into T3 (the effect is due to the activity of the deiodinase enzymes) which is the active form of the hormone on cellular level.

The volume of blood flowing through the thyroid gland in one minute is about five times its weight, indicating intense vascularization. Iodine is the main substrate for the creation of thyroid hormones. Thyroid hormones increase the synthesis of a large number of enzymes, increase the number and volume of mitochondria and affect active membrane transport by increasing the activity of Na+ / K+ ATP enzyme. In conditions of increased metabolism, tissues consume oxygen in larger quantities, and at the same time create more end-metabolic products, which results in vasodilatation and increased blood flow. The minute volume, heart rate and myocardial contractility also increase. The increased consumption of oxygen and the creation of more carbon dioxide results in an accelerated frequency and depth of respiration.

Hyperthyroidism is a condition where the thyroid gland produces and secretes inappropriately high amounts of thyroid hormones, which leads to thyrotoxicosis. There are many different causes of hyperthyroidism, and the most common cause includes Graves' disease (GD), toxic multinodular goiter and toxic adenoma.

This condition impacts many different systems of the body including cardiovascular, respiratory, musculoskeletal, nephro-urinary, gastrointestinal, immune, ophthalmic and reproductive system.

The aim of our study was to establish an experimental animal model of thyroid dysfunction - hyperthyroidism. The deficit of published scientific data regarding experimental animal models in this field of medicine is an obstacle for sufficient experimental data on peripheral effects on organs, tissues and at cellular level in the state of thyroid hormones excess. In the literature there is a limited amount of scientific data about established experimental animal models of thyroid dysfunction. Establishing such experimental models could enable further medical investigations and understanding of the effects of thyroid hormone disturbances.

Material and methods

Twenty Wistar rats with an average age of 7 months were analyzed during the period of 2 months, 50% (10) of females (320 ± 40 gr) and 50% (10) of males (350 ± 40 gr). They were treated for 1 month with pharmacological doses of L-Thyroxin 15μg / 100g body mass in the drinking water and 1 following month without treatment. Before the initial administration of L-thyroxine, the following basic parameters were determined for each rat (body mass index, temperature, thyroid status). The second group of 10 Wistar rats (5 males and 5 females) did not recieve L-Thyroxin and this was the control group used to obtain normal morphometric and biochemical parameters.

All performed procedures were in accordance with accepted European and World regulations for working with experimental animals. The animals were acclimatized at room temperature (18-22 ºC). The light regime was a 12/12 hour day/night cycle (6 am to 6 pm). Access to water was ad libitum, and at the same time the food was balanced with a complete pelleted feed mixture of the KKS 0 type in composition according to the specification of the Schaumann manufacturer.

Group 1 – Hyperthyroid group: 20 Wistar rats (10 males and 10 females), which were treated for 1 month with pharmacological doses of L-Thyroxin 15μg / 100g BM in the drinking water and 1 following month without treatment (follow-up). Before the initiation of the administration of L-thyroxine, the following basic parameters were determined for each rat (under conditions of previous good hydration and nutrition): body mass index (BMI), thyroid status (FT4, FT3), body temperature (ºC). The experiment inclusion criteria were: euthyroid condition for each rat. Once a week and at the end of the experiment, the body mass (BMI) of the animal was determined. The next day after the last administration of L-Thyroxin, the thyroid status was determined for the verification of the achieved overt hyperthyroidism.

Group 2 - Control group: 10 Wistar rats (5 males and 5 females) did not recieve L-Thyroxin and this group was used to obtain normal morphometric and biochemical parameters.

The determination of the thyroid status of the experimental animals through the level of FT4 and FT3 in the serum was carried out by DELFIA immunometric methods on the device 1234 DELFIA®Fluorometer, using commercial kits from the manufacturer (PerkinElmer and Analytical Sciences, Wallac Oy, Finland). The kits were intended for quantitative determination of FT4 and FT3 values in serum. The principle was based on FIA (fluoro-immunometric analysis).

STATISTICAL ANALYSIS AND PRESENTATION OF RESULTS

From the descriptive statistics, the measures of central tendency and variability (mean value, standard deviation) were used. Differences between certain time points as well as differences between groups were analyzed with the Student's t-test for dependent and independent samples and the one-factor and multi-factor analysis of variance ANOVA and MANOVA.

The correlations between the values of the individual parameters of the test were expressed by the Person's correlation coefficient (r). Statistically significant were considered the differences for $p < 0.05$. Statistical series (all defined variables) were shown in tables and figures.The testing of the significance of the differences between two arithmetic means and the dependent samples (in the examined groups) was done with the Student's t-test for dependent samples, and if there was a question of irregular distribution with the non-parametric Wilcoxon test of

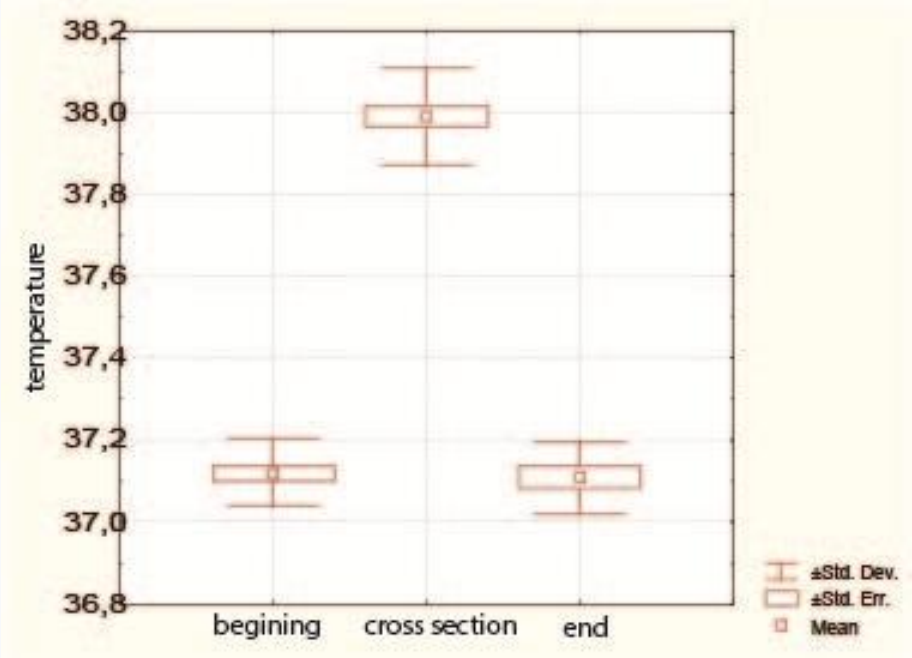
equivalent pairs. The testing of the significance of the differences between two arithmetic means and the independent samples between the test groups was done with the Student's t-test for independent samples and if there was a question of irregular distribution with the non-parametric Mann-Whitney U-test. The data were analyzed with the statistical program SPSS 23.0.

Results

Table 1. Mean values of temperature (^{O}C) at the beginning of the experiment, at cross section and at the end of the experiment in the hyperthyroid group treated with L-Thyroxine

temperature	average	SD	min.	max.	N
beginning	**37.1**	**0.08**	**37.0**	**37.3**	**20**
cross section	**37.9**	**0.12**	**37.8**	**38.2**	**20**
end	**37.1**	**0.08**	**37.0**	**37.2**	**10**

Figure 1. Mean values of temperature (^{O}C) in the hyperthyroid group



There were statistically significant differences between the mean values of the temperatures at the beginning, at cross section and at the end of the study (Friedman ANOVA Chi Sqr. = 16.27; p = 0.00029). Individual differences were tested using the Student's t test for dependent samples.

Table 1A. The significance of the differences in the mean values of the temperatures at the beginning, at cross section and at the end of the experiment in the hyperthyroid group

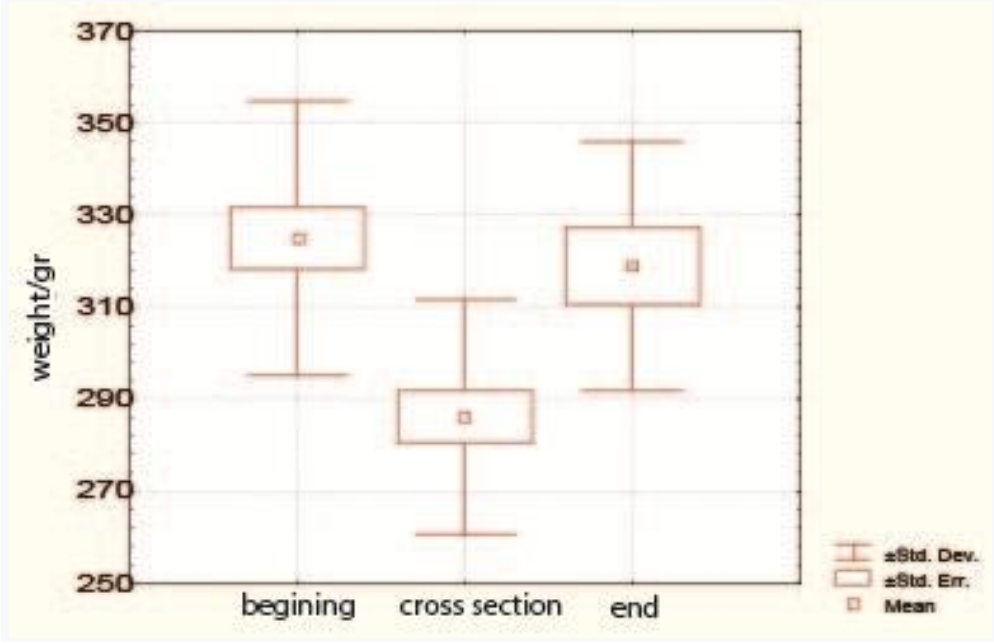
Compared values	Student's t-test (p)
at the beginning - at cross section	t = 31.93 p = 0.000001*
at the beginning - at the end	t = 0.61 p = 0.5554
at cross section - at the end	t = 19.71 p = 0.00001*

* statistically significant difference

Table 2. Mean values of body weight (gr) at the beginning, at cross section and at the end of the experiment in the hyperthyreoid group of subjects.

Body weight	**Average**	**SD**	**min.**	**Max.**	**N**
beginning	**325.0**	**29.64**	**280.0**	**370.0**	**20**
cross section	**286.0**	**25.62**	**250.0**	**340.0**	**20**
end	**319.0**	**26.85**	**280.0**	**350.0**	**10**

Figure 2. Mean values of body weight (gr) at the beginning, at cross section and at the end of the experiment in the hyperthyreoid group of subjects



There were statistically significant differences between the mean values of body weight at the beginning, at cross section and at the end of the study (Friedman ANOVA Chi Sqr. = 16.70; p = 0.00024). Individual differences were tested with Student's t-test for dependent samples.

Table 2A. The significance of the differences in the mean values of the body weight at the beginning, at cross section and at the end of the experiment (hyperthyroid)

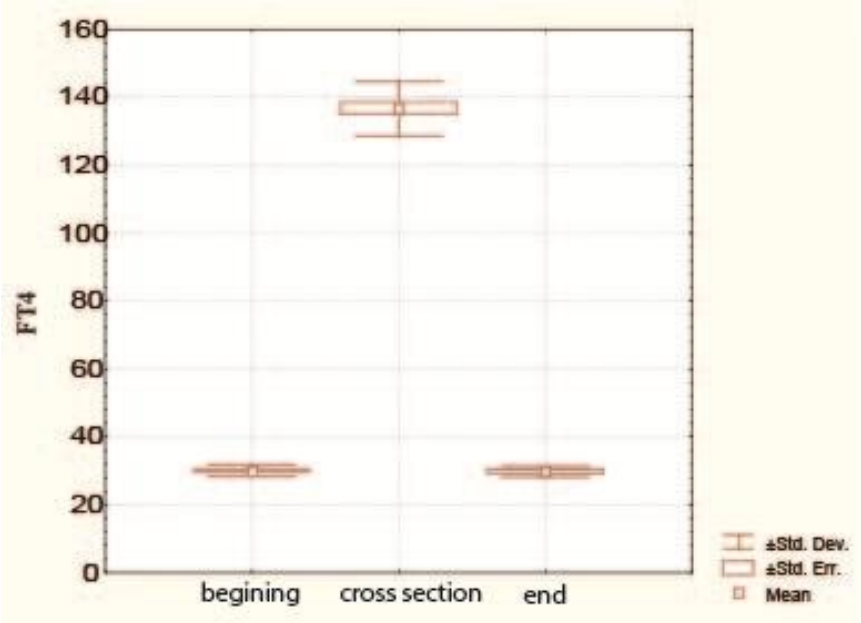
Compared values	Student's t-test (p)
at the beginning - at cross section	t = 15.58 p = 0.00001*
at the beginning - at the end	t = 1.31 p = 0.2228
at cross section - at the end	t = 16.28 p = 0.00001*

* statistically significant difference

Table 3. Mean values of FT4 at the beginning, cross section and at the end of the experiment

FT4	**Average**	**SD**	**min.**	**max.**	**N**
beginning	**30.08**	**1.69**	**27.80**	**33.60**	**20**
cross section	**136.77**	**8.20**	**119.30**	**150.20**	**20**
end	**29.72**	**1.60**	**28.10**	**33.20**	**10**

Figure 3. Mean values of FT4 at the beginning, at cross section and at the end



There were statistically significant differences between the mean values of FT4 at the beginning, at cross section and at the end of the study (Friedman ANOVA Chi Sqr. = 16.80; p = 0.00022). Individual differences were tested with Student's t-test for dependent samples. The differences in relation to FT4 were not significant between the beginning and the end of the examination.

Table 3A. The significance of the differences in the mean values of FT4 at the beginning, at cross section and at the end of the experiment

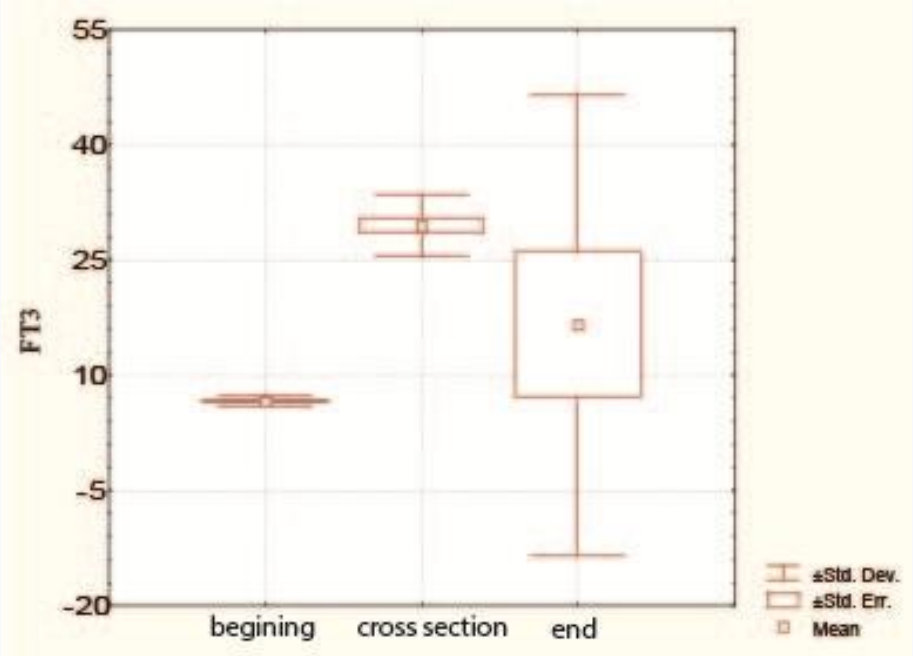
Compared values	Students t test (p)
at the beginning - at cross section	t = 52.37 p = 0.0000001*
at the beginning - at the end	t = 1.19 p = 0.1808
at cross section - at the end	t = 48.81 p = 0.000001*

* statistically significant difference

Table 4. Mean values of FT3 at the beginning, at cross section and at the end (hyperthyroidism)

FT3	**Average**	**SD**	**min.**	**Max.**	**N**
beginning	**6.60**	**0.72**	**5.34**	**7.89**	**20**
cross section	**29.50**	**4.02**	**22.30**	**36.70**	**20**
end	**16.66**	**29.98**	**6.46**	**102.00**	**10**

Figure 4. Mean values of FT3 at the beginning, at cross section and at the end (hyperthyroidism)



There were statistically significant differences between the mean values of FT3 at the beginning, at the intersection and at the end of the study (Friedman ANOVA Chi Sqr. = 18.20; p = 0.00011). Individual differences were tested with the non-parametric Wilcoxon test for equivalent pairs.

The differences in relation to FT3 were not significant between the cross section and the end of the examination.

Table 4A. The significance of the differences in the mean values of FT3 at the beginning, at the intersection and at the end of the experiment (hyperthyroidism)

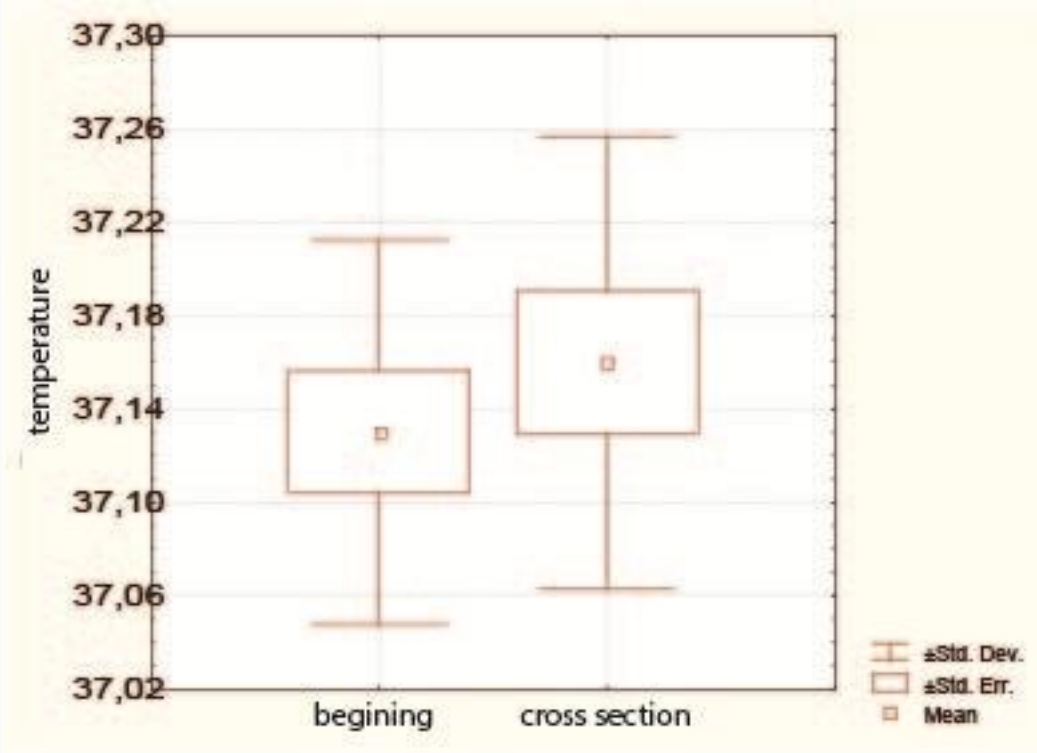
Compared values	Wilcoxon test (p)
at the beginning - at cross section	Z = 3.91 p = 0.000089*
at the beginning - at the end	Z = 2.80 p = 0.00506*
at cross section - at the end	Z = 1.78 p = 0.0744

* statistically significant difference

Table 5. Mean values of temperature (^{O}C) at the beginning and at cross section (control group)

temperature	**average**	**SD**	**min.**	**max.**	**N**
at the beginning	**37.1**	**0.08**	**37.0**	**37.2**	**10**
at cross section	**37.1**	**0.09**	**37.0**	**37.3**	**10**

Figure 5. Mean values of temperature (^{O}C) at the beginning and at cross section (control group)



There were no statistically significant differences (Student's t-test for dependent samples) between the mean values of the temperatures at the beginning and at cross section of the study.

Table 5A. The significance of the differences in the mean values of the temperatures at the beginning and at cross section (control group)

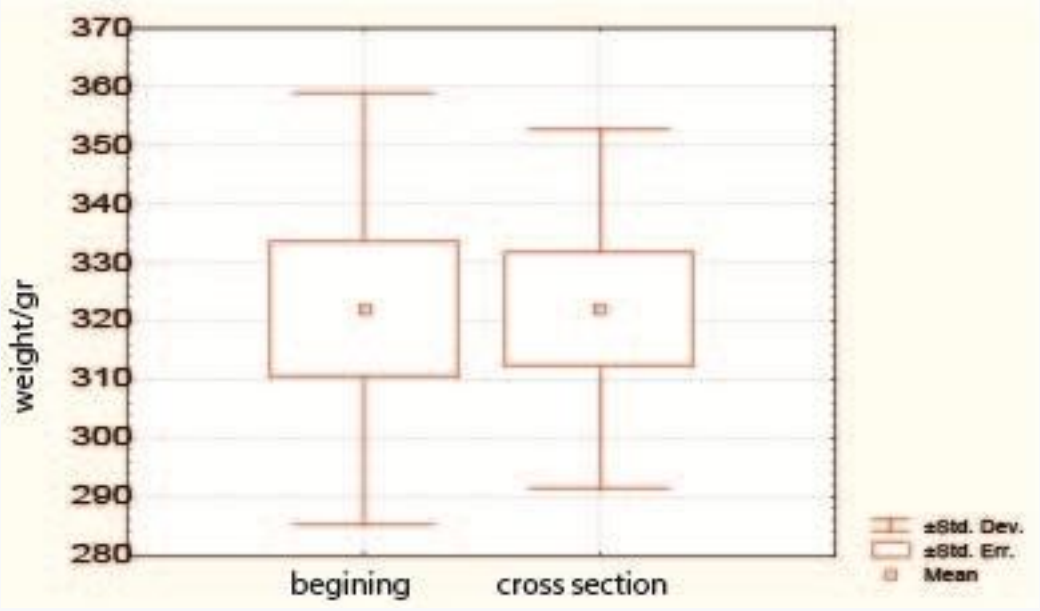
Compared values	Students t test (p)
at the beginning - at cross section	t = 0.63 p = 0.5413

* statistically significant difference

Table 6. Mean values of body weight (gr) at the beginning and at cross section (control group)

Body weight	**Average**	**SD**	**min.**	**max.**	**N**
at the beginning	**322.0**	**36.76**	**280.0**	**360.0**	**10**
at cross section	**322.0**	**30.84**	**290.0**	**360.0**	**10**

Figure 6. Mean values of body weight (gr) at the beginning and at cross section (control group)



There were no statistically significant differences between the mean values of the body weight at the beginning and at cross section of the study (control group without drugs) (Student's t-test for dependent samples).

Table 6A. The significance of the differences in the mean values of the body weight at the beginning and at cross section (control group)

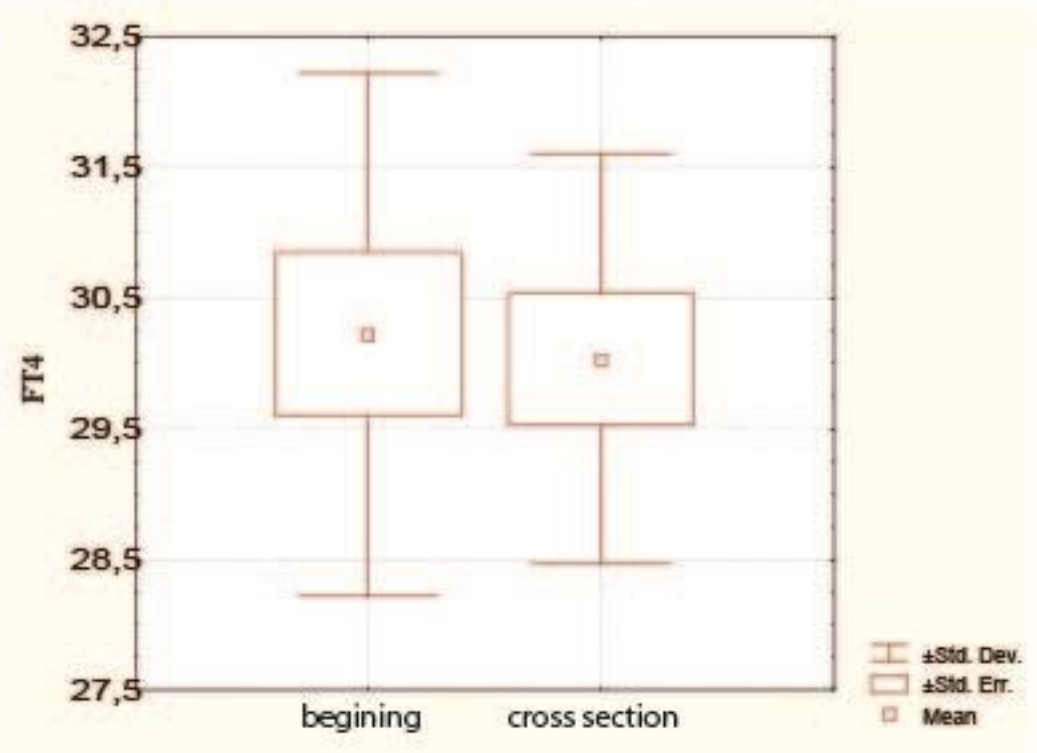
Compared values	Student's t-test (p)
at the beginning - at cross section	t = 0.00 p = 1.0

*statistically significant difference

Table 7. Mean values of FT4 at the beginning and at the cross section (control group)

FT4	**Average**	**SD**	**min.**	**max.**	**N**
at the beginning	**30.22**	**1.99**	**27.60**	**33.70**	**10**
at cross section	**30.03**	**1.56**	**27.80**	**32.70**	**10**

Figure 7. Mean values of FT4 at the beginning and at the cross section (control group without drugs)



There were no statistically significant differences between the mean values of FT4 at the beginning and at the end of the study (control group without drugs) (Student's t-test for dependent samples).

Table 7A. The significance of the differences in the mean values of FT4 at the beginning and at the cross section (control group without drugs)

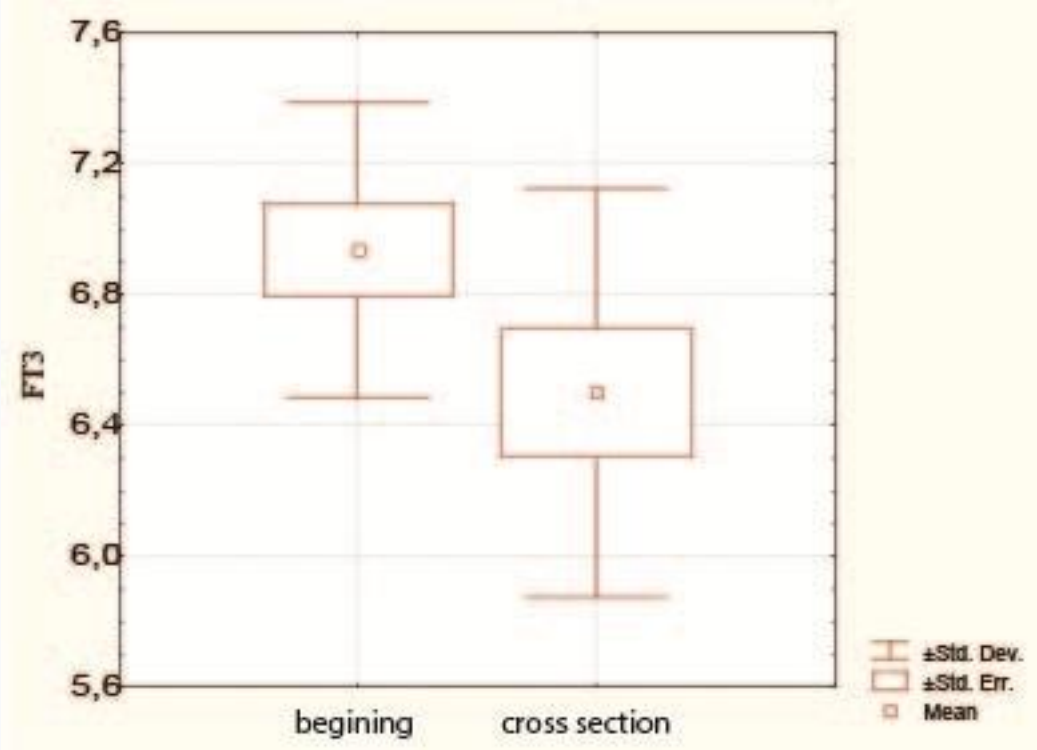
Compared values	Student's t-test (p)
at the beginning - at cross section	t = 0.36 p = 0.7233

* statistically significant difference

Table 8. Mean values of FT3 at the beginning and at the cross section (control group)

FT3	**Average**	**SD**	**min.**	**max.**	**N**
beginning	**6.94**	**0.45**	**6.38**	**7.63**	**10**
at cross section	**6.50**	**0.62**	**5.04**	**7.21**	**10**

Figure 8. Mean values of FT3 at the beginning and at the cross section (control group)



There were statistically significant differences (Student's t-test for dependent samples) between the mean values of FT3 at the beginning and at the end of the study (control group).

Table 8A. The significance of the differences in the mean values of FT3 at the beginning and at the cross section (control group)

Compared values	Student's t-test (p)
at the beginning - at cross section	t = 2.53 p = 0.0321*

*- statistically significant difference

Discussion

Thyroid dysfunction affects metabolic processes in all organs and tissues of the human body. In the literature there is a limited amount of scientific data about established experimental animal models of thyroid dysfunction. Establishing such experimental models could enable further medical investigations and understanding of the effects of thyroid hormone disturbances.

Our experiment included Wistar rats which were treated for 1 month with pharmacological doses of L-Thyroxin 15μg / 100g BM in drinking water. Before the start of the administration of L-thyroxine, each rat was under condition of good hydratiton and nutrition.

Our model was similar to the experimental model of Ferriera *et al.* which was performed in mice [1]. We decided to use the administration of L-Thyroxin in drinking water ad libitum in order to have as little animal disturbance and handling as possible compared to the models of intraperitoneal administration [2,3,4].

Hegazy A *et al.* used a similar experimental model of induced hypothyroidism in adult albino rats but they obtained the blood samples from the retro-orbital venous plexuses and we used the tail veins [5]. Hammad *et al.* used the dosage of 25μg/day, while we obtained overt hyperthyroid state in

our animals at the dosage of 15μg / 100g BM [6]. Several authors obtained positive results of hyperthyroid induction in rats after administering L-thyroxin and confirmed that its action in the organism was dose-dependent [7,8,9].

Thyroid hormones (TH) are of central importance for thermogenesis, energy homeostasis and metabolism. It is widely accepted that THs modulate thermogenesis and body mass directly by changing the functionality and transcription rate of UCP1 and obligatory thermogenesis by increasing metabolic cycling or by direct actions on the sodium/potassium and the calcium pump in skeletal muscle. Hormones of the thyroid gland increase the metabolic activity in all tissues and organs except the brain, retina, spleen, testicles and intestines. Basal metabolism can increase by 60 - 100% above normal values if thyroid hormone levels are high. In our study, the increased internal temperature and changes in the body mass of the rats in the group with induced hyperthyroid state was seen during the cross-section analyses where the levels of thyroid hormones were overtly increased.

There were statistically significant differences between the body temperature and body mass at the beginning, cross section and the end of the study. The amount of thyroid hormones that reaches the peripheral tissues on a daily bases is about 120 nmol of thyroxine and 50 nmol of triiodothyronine. Although the effect of triiodothyronine is four times stronger than that of thyroxine, the length of the effect is four times greater than that of thyroxine in relation to triiodothyronine. In the periphery, thyroxine is almost completely deiodinated into triiodothyronine and therefore it is considered that triiodothyronine is the true intracellular hormone.

Our results showed the levels of thyroid hormones (free triiodothyronine, free thyroxine) to be statistically significantly different between the beginning, cross section and the end of the study, which was in favor of a successfully experimentally induced hyperthyroid state. The levels of FT4 were significantly increased in rats given L-thyroxin in the cross section compared to the euthyroid rats at the beginning or at the end of the study and the control group. T3 levels were also statistically significantly elevated in hyperthyroid rats compared to euthyroid rats at the beginning and at the end of the study and the control group. Serakides *et al*. obtained a positive result of hyperthyroid induction in mice after administering 50 μg of L-thyroxin/animal/day, for 30 days. This dosage given to mice is likely to lead to an intoxication status [10].

Hyperthyroidism has traditionally been associated with weight loss and underweight state. Subjects with hyperthyroidism have an adrenergic hyperstimulation with increased basal metabolism and thermogenesis, and a greater overall energy expenditure resulting in a tendency toward weight loss [11].

Hyperthyroidism can also induce an increased gastrointestinal transit and occasionally anorexia due to the anorexigenic effect of triiodothyronine. All these factors may have led to the belief of a direct association of hyperthyroid states with reduction of body weight. According to the previous knowledge of the effects of hyperthyroidism on the body mass index, the reduction of body weight in our study, since the rats were in hyperthyroid condition, was expected and in favor of a successfully established experimental animal model of thyroid dysfunction - hyperthyroidism.

Our results showed a significantly decreased body weight in the hyperthyroid rats expressed in grams in the cross section compared to euthyroid rats at the beginning and the end of the study and in the control group. The control group of ten rats which were not treated with any drug did not show any statistically significant differences according to the internal body temperature, body mass index or thyroid hormone levels.

Conclusion

Our study confirms that we have successfully established an experimental animal model of impaired function of the thyroid gland - hyperthyroidism.

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