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Research Paper

Evaluation of acute physiological and molecular alterations in surgically developed hypothyroid Wistar rats

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ABSTRACT

Objectives: To explore the general physiological and molecular changes occurring as a result of acute hypothyroidism. **Materials and Methods:** Hypothyroidism was developed by thyroidectomy in wistar rats. After surgery, animals were observed for 14 days in order to determine changes in body weight, feed consumption, rectal temperature, heart rate, and blood pressure, clinical pathological and hormonal alteration. In addition, relative changes in weight, histopathology and MHC – α and β gene expression of heart was also evaluated. **Results:** Thyroidectomised rats showed lethargy, piloerection and decreased locomotors activity. Day dependent significantly decreased body weight and feed consumption were seen in hypothyroid rats. Rectal temperature was significantly reduced at day 7 and 14 after surgery. Heart rate and blood pressure were significantly decreased at day 14 in thyroidectomized rats in comparison with euthyroid rats. Haematological parameters shown high WBC count. Serum LDL and phosphorous levels were high where as triglycerides; total protein, creatinine kinase and globulin were low. Heart weight was significantly high. Histopathology of heart tissue showed myocardial segmental degeneration. Downregulation of MHC – α and upregulation of MHC – β were seen in hypothyroid rats in comparison with euthyroid rats. **Conclusion:** This finding suggests that deficiency of thyroid hormone (TH) in hypothyroidism is associated to a cardiac dysfunction and acute changes in body homoeostasis as result of sudden arrest of thyroid hormone.

Key words: Euthyroid, hypothyroidism, myosin heavy chain, thyroidectomy

INTRODUCTION

Thyroid dysfunction is very common and has a profound effect on a number of metabolic processes in almost all tissues of the body. Thyroid gland releases two types of thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). Hypothyroidism is the most common pathological hormone deficiency. Hypothyroidism can be classified on the basis of its time of onset (congenital or acquired), the level of endocrine dysfunction responsible (primary or secondary, also termed central, hypothyroidism), and its severity (overt [clinical] or mild [subclinical]). Most common signs and symptoms observed during hypothyroidism are fatigue, bradycardia, diastolic hypertension, weight gain and muscle weakness. Hypothyroidism is a risk factor for many cardiovascular diseases and obesity.[1] Changes in cardiac function by TH ultimately depend on the regulation of target genes within the

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heart and indirect effects due to hemodynamic changes by TH. The biologically active thyroid hormone T_3 affects cardiac contractility, heart rate (HR), diastolic function and systemic vascular resistance through genomic and non-genomic mediated effects.[2] Thyroid hormones influence the function of many organs and mediate their diverse actions through two types of thyroid hormone receptors, TRα and TRβ.[3]

Thyroid hormone can limit ischemic injury, attenuate cardiac remodelling and improve cardiac hemodynamics.[4] Thyroid hormone regulates the expression of cardiac MHC – α and β which are primarily responsible for influencing the heart function. Myosin V1, V2 and V3 are isoforms of myosin heavy chain. Replacement therapy with physiological doses of the hormone leads to a shift in distribution of isoenzymes towards V1 (i.e., V3 to V1).[5] T_3 positively regulates MHC – α and negatively regulates MHC – β.[2] Hence, the aim of our work was to describe the methology of surgical hypothyroidism in rat and to evaluate the various physiological alterations in terms of changes in body weight, feed consumption, rectal temperature, heart rate, blood pressure, haematology and serum biochemistry parameters, histopathology of heart and myosin heavy chain alpha and beta gene expression and to assess the similarity of this animal model with the pattern of hypothyroidism in humans.

MATERIALS AND METHODS

Animals

Healthy young nulliparous female Wistar rats 5-7 weeks (130 ± 15 g) obtained from Animal Research Facility of Zydus Research Centre, Ahmedabad, and were housed in Individually Ventilated Cage (IVC) under standard laboratory conditions: Temperature (25 ± 3°C), relative humidity (30 to 70%), photoperiod (light and dark cycle of 12hrs each) with food and water provided *ad libitum*. The protocol of the study was approved by Institutional Animal Ethics Committee (IAEC). All animals were acclimatised for five days prior to starting of experiments. This experiment was conducted in animal research facility at Zydus Research Centre, Ahmedabad.

Physiological evaluation

Totally 20 female wistar rats were divided equally into two groups (Group-1: Euthyroid rats, Group-2: Hypothyroid rats). Combination of 80 mg/kg ketamine and 10 mg/kg xylazine were given by *i.p* route to anaesthetize the animals for thyroidectomy. An anaesthetized and surgically prepared animal was placed in dorsal recumbency. A 2 cm ventral, cervical midline incision was taken with its caudal terminus at the level of the clavicle. The underlying salivary and lymphatic tissue was pushed laterally. Divide omohyoideus muscle and retracted it to visualize the trachea, larynx and thyroid glands. The thyroid gland was gently teased away and removed. Tissues are approximated and a skin incision was closed with nonabsorable suture material (Black Braided Silk). Animals were observed for 14 days for various parameters. 1% calcium gluconate was given to thyroidectomized animals in drinking water for first 10 days, to take care of calcium level. Euthyroid animals were provided food and water *ad libitum*. Animals were observed for 14 days after surgery and the animals were observed for clinical signs and mortality, body weight, food intake, rectal temperature, non-invasive blood pressure and heart rate (Out of 10 animals we had used first six animals from both groups for heart rate and blood pressure measurements) were measured in regular intervals. On day 14th the animals were fasted for overnight and the blood samples were collected form retro-orbital plexus.[6] Whole blood was collected with an anticoagulant 2% di-potassium EDTA. Total Leucocytes Count (TLC), Total Erythrocyte Count (TEC), Platelet Count (PLT), Haematocrit (HCT), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Differential Leukocyte Count (DLC) were analyzed by using Cell-Dyn 3700 haematology analyser (Abott laboratories, USA).

Biochemical analysis was done using Daytona autoanalyser (Randox Laboratories, UK). Details of parameters evaluated and the methods used are as follows: Albumin, total bilirubin, calcium, total cholesterol, high density lipoprotein, low density lipoprotein, creatinine, glucose, phosphorous, total protein, triglyceride, urea, alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), creatine kinase and globulin. Concentration of serum electrolytes such as Sodium, Potassium were analyzed using Instalyte analyser (ERBA, Mumbai, India) by ion-selective method (ISE). Total T_3 (TT_3, catalog No. $T_3$101WB), Total thyroxine (TT_4, catalog No. $T_4$102WB) were estimated.

At the end of the study, all survived animals were humanely euthanised by carbon dioxide asphyxiation and heart was collected and weighed. Part of the heart tissue was fixed in 10% formal saline, their paraffin sections were prepared and Stained with haematoxylin-eosin for histopathological examination.

Gene expression

Out of 10 animals we had used first six animals from both groups for gene expression in heart. Tissue samples from heart were dissected and snap-frozen in liquid nitrogen immediately at terminal necropsy and stored at –70 ± 2°C till further analysis. Equal amount of frozen heart tissue and TRIZOL reagent (1 ml/100 mg of tissue) was homogenized and total RNA (Ribonucleic acid) was isolated. Quantitation of total RNA was performed using Bio-photometer (Eppendorf, Germany) and the quality of RNA was ascertained by agarose gel-electrophoresis. For gene expression of MHC-alpha,

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Myh6F (CACCCTGGAGGACCAGATTA) and Myh6R (TGGATCCTGATGAACTTCCC); and for MHC-beta, Myh7F (TGGCACCGTGGACTACAATA) and Myh7R (CTACAGGTGCATCAGCTCCA) specific RT-PCR (Real Time – Polymerase Chain Reaction) primers were used. First strand cDNA (Complementary deoxyribonucleic acid) synthesis was achieved with 2 µg of total RNA in a final volume of 20 µl. About 2 µl from this reaction cocktail was used directly to conduct PCR amplification in presence of SYBR-Green following real time RT-PCR using ABI-7300 system (Applied Biosystem, Singapore). Ribosomal Acidic Protein was used as internal euthyroid in this study.

Statistical analysis

The data are presented as mean ± SD. Unpaired t test was carried out to find out significant difference between euthyroid and hypothyroid groups. A *P* value <0.05 was considered significant.

RESULT

Hypothyroid rat model was developed by thyroidectomy, in that total T_3 and T_4 were significantly decreased at day 14th after surgery [Figure 1]. No mortality was observed in thyroidectomized rats and the lethargy, piloerection and decrease locomotors activity were observed. The thyroidectomised rat showed significant decrease in body weight compare to euthyroid rat [Table 1]. Day dependent decreasing in feed consumption and rectal temperature was observed in thyroidectomised rats. Feed consumption was found decreased by 38.6%, 47.7%, 49.0% and 54.3% during 1-4, 4-7, 7-11 and 11-14, respectively in thyroidectomised rats in comparison with euthyroid rats. Rectal temperature was found to drop by approximately 2.25 and 2.96°F on day 7 and 14, respectively in thyroidectomized rat in comparison with euthyroid rat.

Heart rate and mean blood pressure were significantly decreased in hypothyroid rat (387 beats/min $^{**}P < 0.01$; 85 mmHg, $^{*}P < 0.05$ respectively) in comparison with euthyroid rat (481 beats/min; 98 mmHg respectively).

Increased total leukocyte count (67.4%), neutrophil count and alteration in erythrocyte index were observed in thyroidectomized rat in comparison with euthyroid [Table 2]. Lipid profile shows marked reduction in triglyceride by 68.4%, high levels of low density lipoprotein by 58.9% and marginal low levels of total cholesterol by 16.1% were noticed in thyroidectomized rat in comparison with euthyroid. Phosphorus, urea and creatinine levels were marginally high by 74.6%, 23.0% and 12.6% respectively in thyroidectomised rat in comparison with euthyroid. Levels of total protein, globulin, and chloride and creatinine kinase were mildly low by 6.2%, 7.6%, 2.7% and 53.7%, respectively in thyroidectomised rat in comparison with euthyroid [Table 3]. Heart weight was found to be significantly decreased by 26% in comparison with euthyroid group [Figure 2]. Myocardial segmental degeneration and MNC cell have observed in hypothyroid rat heart as compared to control rat heart (H and E, ×20) [Figure 3].

MHC – α and β gene expression

Significwant difference in the expression of MHC alpha and beta was observed in hypothyroid euthyroid and euthyroid rats. In euthyroid rats, the MHC α and β are expressed almost in equal proportion, but in hypothyroid euthyroid rats there was

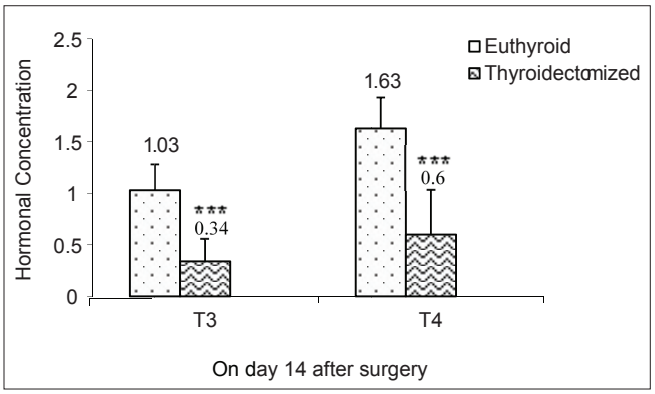

Figure 1: Comparison of levels of total T_3 and T_4 in euthyroid and thyroidectomized rats on day 14th after surgery. ***P<0.05 Vs euthyroid group. n=10 in each group

Table 1: Body weight (g) of rats

Day	Euthyroid rat			Thyroidectomised rat		
	Mean	SD	*N*	Mean	SD	*N*
1	189.42	10.73	10	171.08**	14.01	10
4	195.63	8.30	10	172.56****	11.78	10
7	201.17	9.96	10	172.95****	8.76	10
11	205.18	6.28	10	164.20****	14.37	10
14	205.78	6.30	10	162.87****	15.67	10

**P<0.05 Vs euthyroid, ****P<0.0001 Vs euthyroid; Values are mean±SD

Table 2: Haematological profile of rats

Parameters	Euthyroid rat			Thyroidectomized rat		
	Mean	SD	*N*	Mean	SD	*N*
WBC (10^3/µL)	5.50	0.55	10	9.21*	3.34	10
RBC (10^6/µL)	6.76	0.41	10	6.95	0.44	10
HGB (g/dL)	13.05	0.62	10	12.83	0.82	10
HCT (%)	41.32	1.84	10	40.29	2.61	10
MCV (fl)	61.15	1.52	10	57.99***	1.54	10
MCH (pg)	19.28	0.46	10	18.48***	0.45	10
MCHC (g/dL)	31.55	0.26	10	31.90*	0.33	10
PLT (10^3/µL)	730.50	66.83	10	723.20	135.71	10
NEUT (%)	13.60	4.87	10	16.76	9.71	10
LYMPH (%)	80.32	4.07	10	77.91	11.46	10
MONO (%)	2.46	1.20	10	2.03	1.50	10
EOSIN (%)	1.62	0.43	10	1.64	1.36	10
BASO (%)	2.00	0.52	10	1.66	0.62	10

**P<0.05 Vs euthyroid, ****P<0.0001 Vs euthyroid; Values are mean±SD

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Table 3: Serum biochemistry of rats

Parameter	Euthyroid rat			Thyroidectomized rat		
	Mean	SD	*N*	Mean	SD	*N*
Glucose (mg/dL)	127.03	27.95	10	133.25	22.92	10
Triglyceride (mg/dL)	191.23	82.41	10	60.49***	46.88	10
Cholesterol (mg/dL)	49.93	4.57	10	41.87	12.98	10
LDL- cholesterol (mg/dL)	3.75	1.27	10	5.96*	2.20	10
HDL- cholesterol (mg/dL)	20.23	1.99	10	18.87	6.10	10
AST (U/L)	159.80	51.21	10	148.47	41.37	10
ALT (U/L)	31.35	7.47	10	40.11	14.36	10
Alkaline phosphatase (U/L)	114.88	22.02	10	134.52	69.20	10
Total bilirubin (mg/dL)	0.08	0.05	10	0.10	0.06	10
Total protein (g/dL)	6.73	0.26	10	6.31*	0.47	10
Albumin (g/dL)	3.85	0.14	10	3.65	0.24	10
Urea (mg/dL)	35.63	4.42	10	43.84*	9.28	10
Creatine (mg/dL)	0.69	0.06	10	0.77	0.10	10
Creatine kinase (U/L)	1411.37	568.04	10	653.93***	395.04	10
Phosphorous (mg/dL)	5.60	0.86	10	9.78***	2.29	10
Globulin (g/dL)	2.88	0.13	10	2.66*	0.26	10
Sodium (mmol/L)	142.07	0.86	10	141.81	1.34	10
Potassium (mmol/L)	4.11	0.38	10	3.91	0.38	10
Chloride (mmol/L)	103.77	1.09	10	100.96**	1.89	10

*P<0.05 Vs euthyroid, **P<0.01 Vs euthyroid, ***P<0.001 Vs euthyroid; Values are mean±SD

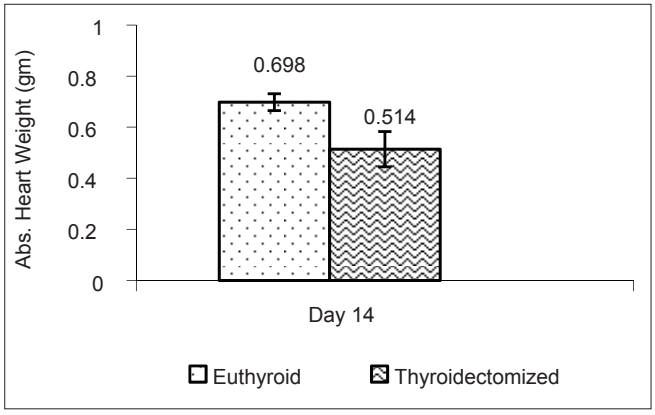

Figure 2: Heart weight of rats; n=10 in each group

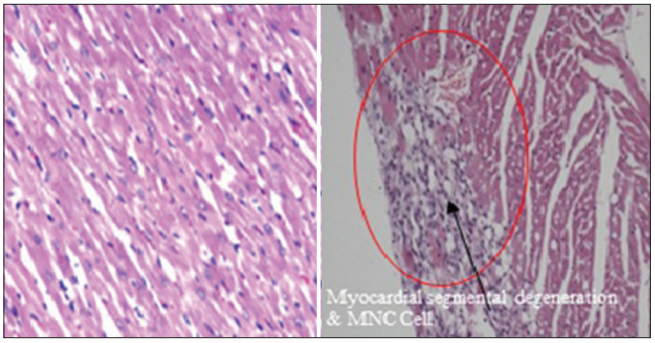

Figure 3: Comparison of normal and hypothyroid cardiomyocytes. Normal cardiomyocytes (H and E, ×20), Hypothyroid heart (H and E, ×20)

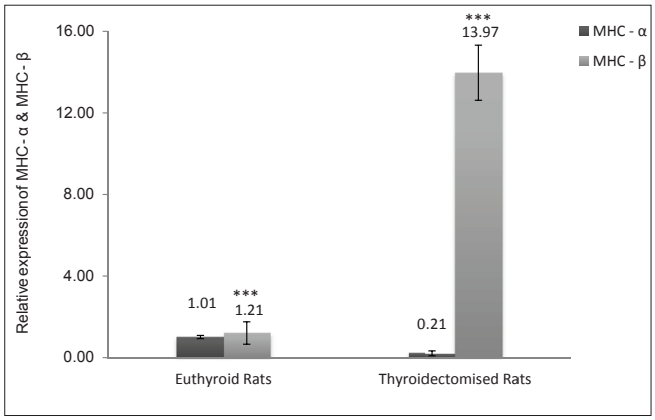

Figure 4: Cardiac MHC– α and ß expression in rats; n=10 in each group; ***P<0.05 MHC-α

marked upregulation of MHC β and downregulation of MHC α [Figure 4].

DISCUSSION

Thyroid hormones (THs) play critical roles in differentiation, growth, and metabolism. Hypothyroidism is the most common pathological hormone deficiency. Endemic cretinism due to iodine deficiency remains a public health problem in developing countries at the advent of the third millennium. Thus the study of deficiency of TH in hypothyroidism has important biological and medical implications. In hypothyroid rats, clinical signs such as lethargy, piloerection and decrease locomotors activity was seen. These effects were well reported with hypothyroidism in various animal studies

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and may be result of decreased basal metabolic rate which leads to decreased cellular metabolism.[7,8]

By real time RT-PCR analysis, cardiac tissue of hypothyroid rat showed changes in MHC isoform expression by marked upregulation of MHC – β gene [11.54 fold higher expression (1054.5%) than euthyroid] and down regulation of MHC–α gene [4.8 fold lesser expression (79.2%) than euthyroid rats]. This alteration in MHC expression is well reported in hypothyroid rat.[9,10] Up regulation of MHC – β gene and down regulation of MHC–α gene indicate that T3 regulates MHC–α positively and MHC-ß negatively [Figure 4].[2]

Due to deficiency of T_3 and T_4 in hypothyroid rats, there is a significant reduction in heart rate (19.5%) and blood pressure (13.9%) in comparison with euthyroid rats. The biologically active TH, T_3 influence the sensitivity of sympathetic system and hemodynamic alterations in the periphery which leads to increased cardiac filling and modification of cardiac contraction.[11,12] Myosin V1 has higher ATPase activity and increased velocity of fiber shortening than myosin V3, so the relative expression of isoenzymes in the heart can determine cardiac contractility. In hypothyroid rats, myosin V3 predominates so the less active myosin subtype participates in the contractile process resulting in decreased velocity of fiber shortening. In contrast, T_3 treatment stimulates a-MHC gene expression and decreases b-MHC gene expression, leading to increased myosin V1, and enhanced cardiac contractility.[13]

Decreased heart weight by 26% in hypothyroid rats in comparison with euthyroid rats indicates the sensitivity of T_3 with myocardial cell to catecholamines and/or its effect on hemodynamic changes [Figure 2].[11-14] Gene expression study also indicates marked downregulation of MHC-α: MHC-β ratio may indicate inhibition of cardiac TRα receptor which is responsible for diminish the synthesis of myocardial contractile protein, which ultimately lead to atrophy of heart and decreased cardiac weight.

In chronic hypothyroidism, body weight is gain but in our study due to suddenly hormonal disturbances significantly decline in bodyweight was seen in hypothyroid rats in comparison with euthyroid rats. It is well reported that hypothyroid rats are hypophagic and do not gain weight rapidly.[15,16]

Due to deficiency of T_3 and T_4 in thyroidectomized rats decreased in feed consumption was found in comparison with euthyroid rats indicates deficiency of T_3 decreased breakdown of macronutrient via glycogenolysis and gluconeogenesis as a result of decreased BMR. Decreased appetite is associated with decreased in metabolic rate, so less energy is required to maintain the equilibrium of decreased demand of energy. In addition, deficiency of T_3 causes decreased in hypothalamus AMPK (AMP–activated kinase) activity which inturn contribute to the development of deficiency of T_3 induced hypophagia. AMP–activated kinase is a protein kinase that is activated when cellular energy is depleted.

Decrease in body temperature in thyroidectomized rats due to deficiency of T_3, because T_3 has calorigenic effect which maintains a critical role in the euthyroid of body temperature by stimulation of thermogenesis and regulation of cellular metabolism.[7]

Effect of Hypothyroidism on metabolic profile was evident through determination of various serum biochemical analytes in this study Triglyceride level was significantly decreased by 68.4% in hypothyroid rats and this is due to decreased feed consumption in hypothyroidism. LDL was seen by 58.9% higher in thyroidectomised rats in comparison with euthyroid. This is due to decreased in hepatic LDL receptor mRNA to promote the LDL clearance process. In hypothyroidism there is a decreased in BMR and is also responsible for increased LDL level.[17,18] Total protein levels were significantly low with relative decline in albumin and globulin levels. Creatinine kinase was significantly decreased in thyroidectomized rats in comparison with euthyroid rats. It has been reported that serum creatinine kinase level significantly decreased in hyperthyroidism condition [Table 2].[19]

Microscopic examination of hypothyroid heart tissue revealed myocardial segmental degeneration and mononuclear cell infiltration (MNC). This shows cardiac damage in hypothyroidism [Figure 3].

CONCLUSION

In conclusion, the present study offers a further contribution to the growing evidence that deficiency of thyroid hormone (TH) in hypothyroidism is associated to a cardiac dysfunction and suggests acute changes in body homoeostasis as result of sudden arrest of thyroid hormone, which is fully reversible by L-T_4 administration.

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