

# The Influence of Hypothermia on Thyroid Function in Rats

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**T**he study was designed to investigate the influence of hypothermia on thyroid gland function and its role in metabolic balances.

**Materials and Methods:** Superficial hypothermia, bringing the body temperature to 25°C, was induced in ten rats (albino, Wistar race) with a mean age of 8 months. Serum levels of FT<sub>3</sub>, FT<sub>4</sub>, T<sub>3</sub>, T<sub>4</sub>, and TSH were determined before and just after hypothermia, repeated every 24 hours for 4 days.

**Results:** Hormone levels, measured by radio-immunoassay, changed during the study. On different days of study, TSH levels altered, although not significantly, from basal values. Other hormones, decreased significantly after hypothermia, except for T<sub>3</sub> that increased significantly on day 3 compared to basal levels. FT<sub>3</sub> and FT<sub>4</sub> showed the most decrease. Serum T<sub>4</sub> level decreased significantly until 48 hours following hypothermia, after which the decrease was not significant and thereafter began to rise and return to basal levels. Although the body temperature of rats decreased significantly after hypothermia, it increased the day after hypothermia and reached closer to body basal temperature (37°C) after 96 hours.

**Conclusion:** Our findings indicate reduced activity of the thyroid gland and hypothalamus-pituitary axis during hypothermia, this being more prominent in the thyroid gland.

**Key Words:** thyroid gland function, hypothermia, Wistar rat.

## Introduction

The thyroid hormones exert various effects on cardiovascular function.<sup>1</sup> Differing states of disease verify these influences.<sup>2</sup> Heart rate, stroke volume and contractile force, which are results of imbalance in enzymatic function and alterations in protein synthesis,<sup>4</sup> decrease in hypothyroidism.<sup>3</sup> This evidence shows a significant association between thyroid hormones and cardiac function. Studies show that cardiopulmonary bypass (CPB) and hypothermia result in noticeable decrease in circulatory thyroid hormones.<sup>5</sup> Muller et al. revealed that cardiopulmonary bypass with hypothermia alter thyroid hormones in a way that TSH increases during operation but normalizes during first postoperative day. T<sub>3</sub> also decreases and returns to normal after several days.<sup>5</sup> Mitchell et al. reported that in infants undergoing CPB, T<sub>3</sub>, T<sub>4</sub> and TSH decrease while FT<sub>4</sub> increases.<sup>6</sup> Studying infants, Mainwaring et al. showed that FT<sub>4</sub> increases transiently in CPB and hypothermia while T<sub>3</sub>, T<sub>4</sub>, TSH and TRH decrease dramatically immediately following the operation.<sup>7</sup> After 5 days, influenced by TSH, serum levels of T<sub>3</sub>, FT<sub>3</sub> and T<sub>4</sub> returned to normal values. They

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suggested that concurrent CPB and hypothermia give rise to a transient attenuation of pituitary-thyroid axis in infants. The aim of this study is to determine the influence of hypothermia per se, without the resultant stress associated with Cardiopulmonary bypass.

## Materials and Methods

Ten male rats (albino, Wistar) with an average weight of 285 gr. and age of 8 months were enrolled. To induce hypothermia, we used a device designed in the physiology department of Tabriz University of Medical Sciences. The rats after being anesthetized were placed in a specific section of the device and the desired temperature was set. The device had a sensor connected to a thermostat at one end and to the rat rectum at the other. The sensor was also connected to a digital screen, displaying the body temperature of the rat throughout the procedure. First, rectal temperature of the animals was determined. General anesthesia was induced by sub peritoneal injection of chloral hydrate (50 mg/100 gr), following which 2mL of blood was withdrawn from the heart. Body temperature was measured rectally and the rats were placed in the hypothermia device, the temperature of which had initially been set to 37°C. The temperature was then gradually decreased to 25°C, at which temperature the animals were kept for 2 hours. Following this, blood samples were taken as before. After 24 hours a transient state of drowsiness was induced in the rats by placing the rats with ether-soaked swabs in desiccators and removing them immediately following induction of drowsiness for blood sampling, which was repeated every 24 hours for 96 hours.

To separate sera, all blood samples were centrifuged (2500 rpm, 20 min). Serum samples were kept in paraffin-sealed test tubes and stored at -22°C. Thyroid hormones were measured by radio-immuno assay kits (Kavoshyar Co, Iran) at the hormone laboratory of Imam Khomeini hospital in Tabriz. The hormone levels before induction

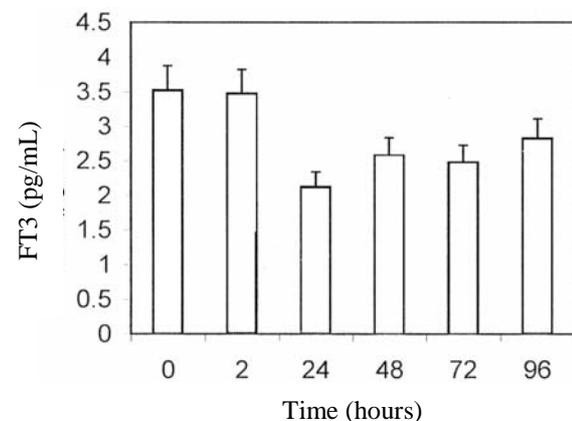
of hypothermia (time 0) were considered as the basal levels, which were then compared with the levels obtained, after induction and maintenance of hypothermia, using SPSS software (Windows), and the paired t-test.  $P < 0.05$  was considered significant.

## Results

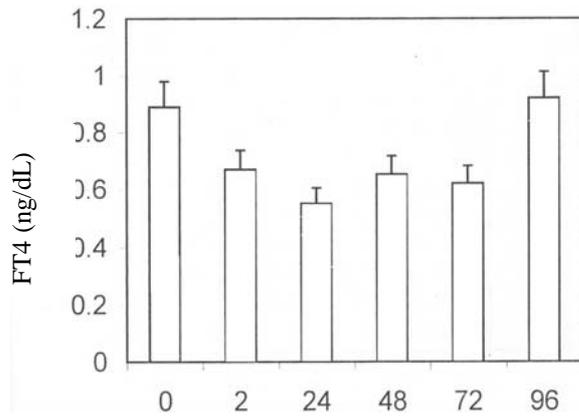
In this study investigating the influence of hypothermia per se, without the effects of stress associated with CPB or cardiac arrest on the thyroid gland activity in the animal models, our findings were as follows:

### FT3:

Basal level of FT3 in the physiologic state was  $3.53 \pm 0.219$  pg/mL, decreasing after induction of hypothermia and reaching its nadir during the 24 hours following hypothermia ( $2.12 \pm 0.28$  pg/mL) significantly lower than physiologic levels ( $p < 0.002$ ). FT3 level, then, increased gradually, but it was significantly lower than in the physiologic state during the first 78 hours after hypothermia, reaching  $2.83 \pm 0.204$  pg/mL after 96 hours which was not significantly different from the basal level. (Fig.1)



**Fig. 1. Serum FT3 levels before (0) and just after hypothermia (2 hours) 24, 48, 72, and 96 hours after hypothermia.**



**Fig. 2.** Serum FT4 levels before (0) and just after hypothermia (2 hours) and 24, 48, 72, and 96 hours after hypothermia.

#### **FT4:**

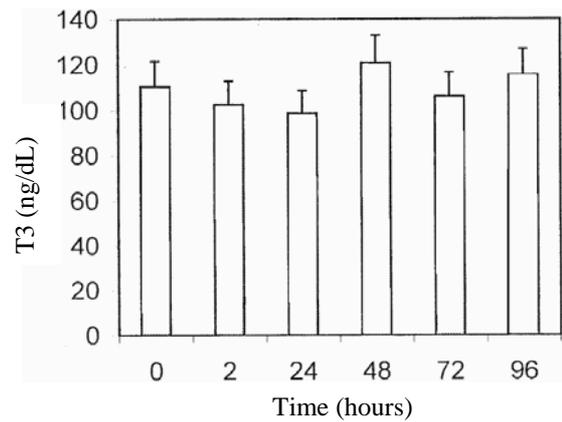
Before hypothermia the basal level of FT4 was measured at  $0.89 \pm 0.042$  ng/dL, compared to which, significant differences in levels of FT4 were observed just after hypothermia, the differences remaining significant for 96 hours, during which time FT4 level reached  $0.68 \pm 0.08$  ng/dL ( $p < 0.016$ ) (Fig. 2).

#### **T3:**

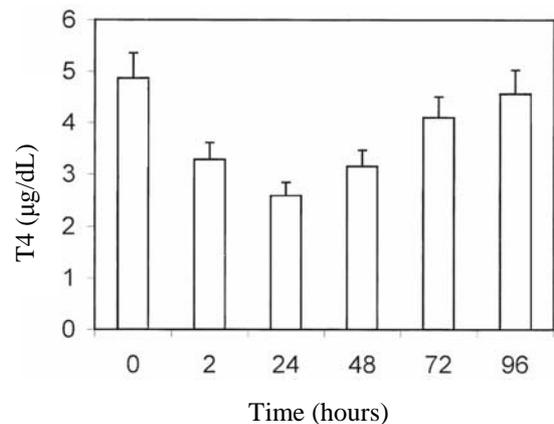
Prior to hypothermia, the basal level of T3 was  $110 \pm 13.59$  ng/dL, its mean level showing no significant difference from basal levels in the 24 hours following hypothermia. It however increased to  $121 \pm 14$  ng/dL after 48 hours, which was significantly higher than basal levels ( $p < 0.045$ ). After 72 hours, it decreased and reached  $115 \pm 8$  ng/dL 96 hours after hypothermia, which did not differ significantly from basal levels (Fig.3).

#### **T4:**

Basal T4 level was  $4.87 \pm 0.59$   $\mu$ g/dL. Significant alteration of T4 level was seen just after hypothermia lasting for 48 hours after hypothermia ( $p < 0.014$ ). After 48 hours T<sub>4</sub> levels began to increase and reached  $4.57 \pm 53$   $\mu$ g/dL 96 hours after hypothermia (Fig. 4).



**Fig. 3:** Serum T3 levels before (0) and just after hypothermia (2 hours) and 24, 48, 72, and 96 hours after hypothermia.



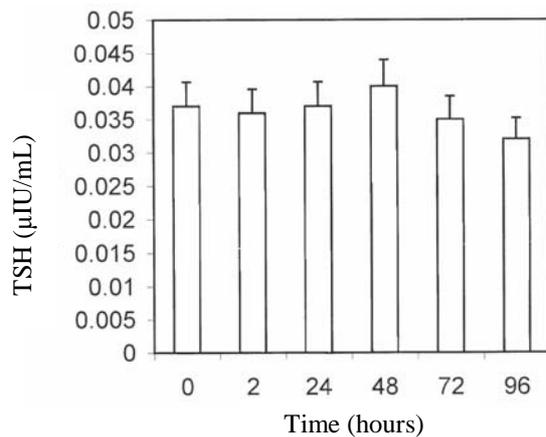
**Fig. 4:** Serum T4 levels before (0) and just after hypothermia (2 hours) and 24, 48, 72, and 96 hours after hypothermia.

#### **TSH:**

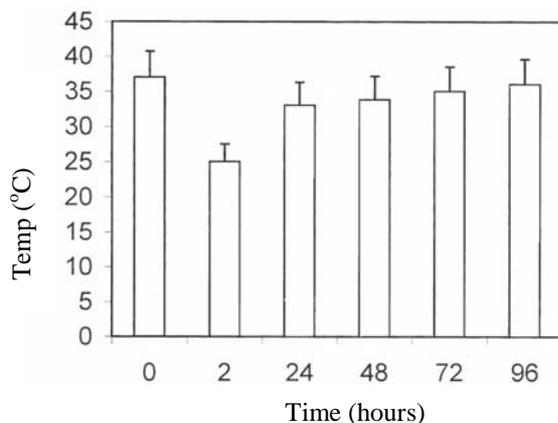
Basal TSH level, prior to hypothermia, was measured at  $0.037 \pm 0.002$   $\mu$ IU/mL and although alterations were seen within first 96 hours after hypothermia, they were non-significant (Fig. 5).

#### **Alterations of temperature:**

Body temperature is normally  $37 \pm 0.5^\circ\text{C}$  and body temperature during hypothermia



**Fig. 5:** Serum TSH levels before (0) and just after hypothermia (2 hours) and, 48, 72, and 96 hours after hypothermia.



**Fig. 6:** Mean body temperature of rats before (0) and just after hypothermia (2 hours) and 24, 48, 72 and 96 hours after hypothermia.

was  $25 \pm 0.5^\circ\text{C}$ . The body temperature of the rats was seen to decrease after hypothermia followed by an increase toward normal body temperature during the following days, eventually reaching  $36 \pm 0.22^\circ\text{C}$ , 96 hours after hypothermia. Body temperature of rats differed significantly from normal values during this period ( $p < 0.001$ ) (Fig. 6).

## Discussion

This study investigates the influence of hypothermia on thyroid function in animal models. Our findings indicate remarkable

alterations in thyroid hormones, in a way that serum levels of FT3 and T4 decreased dramatically compared to basal values, during and after hypothermia. T3 level did not change within 24 hours of hypothermia, while it increased significantly compared to the levels measured before hypothermia. TSH level was seen to alter during the study, though its alterations were not significant.

Our findings indicate that serum T<sub>4</sub> level diminished after hypothermia, reaching its nadir 24 hours thereafter. It then increased to approximate its basal level in 96 hours. The decrease of serum T<sub>4</sub> level, seen in the studies of Mitchell and Mainwaring, was considered to be an effect of hypothalamus-pituitary-thyroid axis attenuation<sup>6,7</sup>. In this study, serum TSH level, the stimulator of thyroid hormones synthesis and secretion, did not differ significantly. Thyroid hormones are hormones most stored in the body, a storage that could easily prevent thyroid failure for a long time, and thereby be expected to maintain normal serum T<sub>4</sub> level. This was not the case in and thyroid hormones decreased significantly. This study on the other hand, it has been reported that in hypothermia, serum norepinephrine levels increase to 74 times their normal levels<sup>8</sup>. As norepinephrine increases the formation of endocytic cysts from droplets of follicular constituents as well as the secretion of thyroid hormones,<sup>8</sup> drastic decrease of circulating T<sub>4</sub> indicates the inability of T<sub>4</sub> to be released from its source. It shows that hypothermia probably affects the secretion of T<sub>4</sub> directly. Another reason could be the excessive conversion of T<sub>4</sub> to T<sub>3</sub> under the stressful conditions resulting from hypothermia. Unlike T<sub>4</sub>, on the third day, T<sub>3</sub> showed a significant rise in comparison to basal levels ( $p < 0.045$ ).

Several studies have shown serum T<sub>3</sub> to decrease postoperatively in hypothermia, returning to preoperative basal values within 3 to 5 days.<sup>6,7,9,10</sup> The investigators considered the fall in T<sub>3</sub> level to be a result of decreased T<sub>3</sub> production in its peripheral source (e.g.

liver), stress and acute illness, a defect in the hypothalamus-pituitary axis, reduction of the activity of 5'-deiodinase (type 2) and low secretion of TSH. In this study, the alterations in TSH levels are in contrast to the findings of other studies. Various factors may be responsible for this difference. For instance, in rats, circulatory T<sub>3</sub> results from the conversion of T<sub>4</sub> to T<sub>3</sub> in thyroid gland by 5'-deiodinase (type 1),<sup>12</sup> while 5'-deiodinase type 1 mediates the conversion of T<sub>4</sub> to T<sub>3</sub> in peripheral tissues.<sup>13</sup> Type 1 5'-deiodinase is specifically activated by TSH while 5'-deiodinase type 2 is not under the influence of TSH and acts more by stress, other peripheral factors and the amount of available substrate (T<sub>4</sub>).

Considering the aforementioned details, we may presume that hypothermia increases type 1 enzyme activity by an unknown self-regulating mechanism in a way that despite decreased secretion of T<sub>4</sub>, serum T<sub>3</sub> remains constant. The process may reduce serum T<sub>4</sub> further, because T<sub>3</sub> production is still normal in spite of low absorption of follicular fluid, which results in low T<sub>4</sub> production. The organism probably compensates for the low metabolism following hypothermia by preventing the reduction of a more metabolically potent hormone, T<sub>3</sub>.

FT<sub>4</sub> began to decrease significantly following hypothermia, a decrease that was significant even after 4 days of hypothermia. A similar decrease was also observed in FT<sub>3</sub>, persisting for the 3 following days. Some earlier studies have reported an increase of FT<sub>4</sub>. According to different researchers, different factors such as addition of heparin to FT<sub>4</sub> in test tubes, decreased binding of protein to the hormone due to CPB and hypothermia are thought to have contributed to increase of FT<sub>4</sub>.<sup>7</sup> Previous studies have also reported a reduction in FT<sub>3</sub>.<sup>7-9</sup>

Bremmer et al. and Mainwaring et al. considered the low conversion of T<sub>4</sub> to T<sub>3</sub> to be the cause of low FT<sub>3</sub> levels owing to the decreased activity of 5'-deiodinase.<sup>7-9</sup> Our findings differ from the two previous studies,

which may be a result of not using heparin in the present study. Also, frozen plasma was not used in this study. Both of these factors cause a false increase of FT<sub>4</sub>. It could, therefore, be said that our study shows the direct effect of hypothermia on FT<sub>4</sub> and FT<sub>3</sub> levels without being influenced by artificial phenomena. When secretion of T<sub>4</sub> from thyroid is low and thyroxin binding globulin level (TBG) does not change during hypothermia (as in other plasma proteins), a balance exists between free and protein bound forms of hormone, keeping FT<sub>4</sub> at a low level, because in this situation, low T<sub>4</sub> could not perform its buffer role to keep the level of free active form of hormone.

Regarding the decrease in FT<sub>3</sub>, despite increased T<sub>3</sub>, this may be due to more uptake of FT<sub>3</sub> by peripheral cells and definitely requires further investigations and more studies. TSH alterations during and after hypothermia in this study are not consistent with those of previous investigations. Previous studies have shown that TSH decreases significantly as compared to basal values before CPB and hypothermia. Investigators have suggested various factors as being responsible for the low secretion of TSH.

Mainwaring et al. considered these phenomena to be a result of FT<sub>4</sub> increase or steroid injection preoperatively leading to decreased secretion of TSH.<sup>7</sup> They also believe that anesthesia, the severity of hypothermia, the duration of cardiac arrest and the perfusion procedure in CPB may contribute to hypothalamus-pituitary-thyroid axis response. In this study, TSH level did not alter significantly while FT<sub>4</sub> and T<sub>4</sub> decreased significantly. Normally in the case of a healthy hypothalamus-pituitary axis, its activity leads to a rise in TSH levels. On the other hand, since in hypothermia, norepinephrine levels increase to 4-7 times their normal levels, this can increase TRH secretion through  $\alpha$ -adrenergic receptors in the paraventricular nucleus (PVN), leading to a rise of TSH synthesis and secretion.<sup>16</sup>

Hypothermia seems to partially impair the hypothalamus-pituitary-thyroid axis function, an impairment that does not, however, suppress TSH secretion completely, but restricts secretion of the hormone amounts.

Overall, hypothermia results in limited serum levels of thyroid hormones, which we believe is due to decreased activity of the thyroid gland and the hypothalamus-pituitary axis, more so in the former. Further investigations measuring TRH, rT3 and related enzymes are deemed necessary to confirm the results of this study.

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