INTRODUCTION

Seasonal changes and environmental temperatures affect hormonal responses and cognition in movements.¹ The body responds by heat production and heat conservation when exposed to prolonged cold environment.² Efficient thermogenesis is achieved by thyroid hormones.¹,³ These help in the central regulation of body temperature through hypothalamus and also in the peripheral regulation by changing cellular metabolism.¹ Studies have shown a significant rise in the thyroid hormones level when rats were exposed to cold temperatures for long durations.⁴ Normally, oxidants are produced in the cells during metabolic activity and are combated by intracellular antioxidants.⁵ In conditions of increased stress like in smokers, older patients or persons experiencing environmental stress from heat, cold or radiation, the production of free radical species is dramatically increased.⁶ In these conditions, the oxidant-antioxidant balance of the body is disturbed. Thyroid hormones are associated with the oxidative and antioxidative status of the body due to its role in oxidative metabolism.⁷ Cold exposure increases the production of reactive oxygen species (ROS) which are associated with cellular damage.⁸ Therefore, the requirement for antioxidants is amplified and without exogenous intake, it becomes difficult to prevent oxidative damage to the cells.⁹ Antioxidant defense systems activate free radical scavengers to protect the body against oxidative injury.¹⁰ Ascorbic acid is one of an important water-soluble antioxidants. Studies have shown that vitamin C has protected the thyroid acinar from oxidative damage and has aided in restoring thyroid hormones’ synthetic function.⁷ As an antioxidant, primary role of vitamin C is to neutralize free radicals.¹¹ Since ascorbic acid is water soluble, it works both inside and outside the cells to fight against free radical damages.⁵ Free radicals seek for an electron pair to regain their stability. Vitamin C is an excellent source of electrons to counter and neutralize the radicals.¹²

The aim of the present study was to compare the thyroid hormone levels in cold exposed rats with and without supplementation with ascorbic acid.

METHODOLOGY

This study was carried out at the Physiology Department of Islamic International Medical College, Rawalpindi, National Institute of Health, Islamabad and Railway Hospital, Rawalpindi, from January to December 2009.

It was an experimental study. Nine weeks old healthy, male Sprague-Dawley rats, weighing 200 ± 25 grams were included in this study. Diseased rats or those which...
developed any disease during the study period were excluded.

Ninety healthy Sprague-Dawley rats (N = 90) were randomly divided into three groups with 30 rats in each group.

Group-I (control group) rats were fed on standard diet and kept at room temperature at 22 ± 3°C.8

Group-II (cold exposed group) was fed on standard diet. They were exposed to cold environment between 8 – 14°C for 1 h/day for one month by keeping the cages in ice-filled tubs and recording the temperature by thermometer.8

Group-III (cold exposed with ascorbic acid supplementation group) rats were fed on standard diet. They were additionally given ascorbic acid (Vitamin C Ascorbic acid Merck, research grade Cat No. 500074) supplement in a dose of 500 mg/L mixed in drinking water.11 They were also exposed to cold environment as for group-II.

After one month, the rats were sacrificed and intra-cardiac blood sampling was done. The blood samples were collected in gel tubes and were centrifuged at a speed of 4000 rpm for 5 minutes to separate the serum. This was analyzed for serum total tri-iodothyronine (T3), thyroxin (T4) and thyroid stimulating hormone (TSH) by using chemiluminescent immunometric assay on Siemens Immulite 2000 Analyzer.5

Data was analyzed by Statistical Package for Social Sciences (SPSS) version 16.0. Mean and standard deviation was calculated for all the numerical data including serum T3, T4 and TSH levels in all the three groups. The statistical significance of differences across the groups was determined by applying One Way ANOVA test with Tukey's Post Hoc test. A p-value ≤ 0.05 was considered significant.

**RESULTS**

Serum T3 levels were significantly different in control group, cold exposed group (47.35 ± 2.21 vs. 51.72 ± 6.81 vs. 50.92 ± 5.73 ng/dL, p = 0.004) and cold exposed with ascorbic acid supplementation group.

Serum T4 levels were also significantly (p = 0.002) different in control group (1.92 ± 0.47 µg/dL), cold exposed group (2.41 ± 0.58 µg/dL) and cold exposed with ascorbic acid supplementation group (2.09 ± 0.52 µg/dL).

The difference in serum TSH levels were also found highly significant (p < 0.001) in (0.16 ± 0.03 µIU/L) control group, cold exposed group (0.38 ± 0.13 µIU/L) and (0.29 ± 0.04 µIU/L) in the cold exposed with ascorbic acid supplementation group as given in Table I.

Multiple comparisons of all three groups among each other as given in Table II, showed that the cold exposed (group-II) and the cold exposed with ascorbic acid supplementation (group-III) both had significantly (p < 0.05) elevated levels of serum T3 as compared with control (group-I). But there was no significant difference in group-II and group-III (p = 0.827). Comparison of serum T4 levels showed that there was significant (p = 0.001) difference in group-I and group-II. Similarly, there was significant difference between group-II and group-III (p = 0.048) with respect to serum T4 level.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Cold exposed group</th>
<th>Cold exposed with ascorbic acid supplementation</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum T3 (ng/dl)</td>
<td>Control group</td>
<td>47.35</td>
<td>Cold exposed group</td>
<td>30</td>
<td>47.35</td>
<td>2.21</td>
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<tr>
<td></td>
<td>Cold exposed</td>
<td>51.72</td>
<td>Cold exposed with ascorbic acid supplementation</td>
<td>30</td>
<td>51.72</td>
<td>6.81</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>50.92</td>
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<td>Serum T4 (µg/dl)</td>
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<td>Cold exposed group</td>
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<td>0.47</td>
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<td>2.41</td>
<td>Cold exposed with ascorbic acid supplementation</td>
<td>30</td>
<td>2.41</td>
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<td></td>
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<td>2.09</td>
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<td>Serum TSH (µIU/ml)</td>
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<td>Cold exposed group</td>
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<tr>
<td></td>
<td>Cold exposed</td>
<td>0.38</td>
<td>Cold exposed with ascorbic acid supplementation</td>
<td>30</td>
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<td>0.13</td>
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<td>0.29</td>
<td>0.04</td>
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* Significant at 5% level of significance

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>(I) group</th>
<th>(J) group</th>
<th>Mean difference (I - J)</th>
<th>p-value</th>
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<td>0.827 **</td>
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<td>Cold exposed with ascorbic acid supplementation</td>
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<td>&lt; 0.0001</td>
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</table>

*The mean difference is significant at the 0.05 level; **The mean difference is insignificant.
Both groups had elevated levels as compared with controls (group-I). Serum $T_3$ levels were not significantly (p = 0.411) different in group-I and group-III. According to the results, group-II and group-III both had significantly (p < 0.05) lower levels of TSH as compared with controls (group-I). Similarly, TSH level was significantly (p = 0.000) greater in group-II in comparison with group-III as given in detail in Table II.

**DISCUSSION**

The present study was planned to observe the effects of antioxidant (ascorbic acid) supplementation on thyroid hormone levels after prolonged cold exposure.

In this study, the serum $T_3$ and $T_4$ levels increased markedly in the cold exposed group as compared to the control group. A study conducted in Japan found an increase in serum $T_3$ and $T_4$ levels suggesting stress caused by cold exposure.8

Eastman and colleagues showed the response of thyroid hormone to prolonged cold exposure in man.13

The subjects in their study spent 4 days in a cold room at 6.6°C. The results were a rapid increase in the concentration of $T_3$ and a slower rise in $T_4$ concentration.

Comparable results were obtained by Venditti et al. who investigated the role of iodothyronine in liver mitochondria of rats after cold treatment.14 They observed hyperthyroidism along with swelling of mitochondria due to overproduction of ROS. In another study, elevated levels of serum $T_4$ and TSH were found in naked mole-rats after 18 months of cold exposure at 25°C.1 The present results are comparable to the results of above studies.

However, Arruda et al. did not get results consistent with the present findings.2 They observed a significant decrease in serum $T_4$ levels while serum $T_3$ levels remained as such in the cold exposed group as compared to the control group of rabbits. It was interpreted that cold exposed rabbits consumed serum $T_4$ to generate $T_3$. The difference of these results from ours might be due to species difference which suggests that thermogenesis during cold temperatures in rabbits is not solely dependant on thyroid but in part depends on skeletal muscles and other processes involving oxidative metabolisms.

A study conducted in Nigeria showed results contrary to the findings of the present study.15 They exposed female Sprague-Dawley rats to 18 – 19°C for 6 weeks. They observed a lower core body temperature along with decreased serum $T_4$ levels in the cold exposed group than the control group. However, serum TSH levels were raised in the cold exposed group, may be due to a reduction in negative feedback on the hypothalamic-pituitary-thyroid axis as a result of the fall in serum $T_4$. This difference in results may be because of geographical difference as the study was conducted in Nigeria and also because of experimental animal’s gender difference. Females may show a different behaviour to thermogenesis because of hormonal variation.

The third group in this study was of rats which were exposed to cold environment along with ascorbic acid supplementation. They showed a reduction in serum $T_3$, $T_4$ and TSH levels. These values, however, remained higher than the control group. A study conducted by Ladmakhi and fellows gave comparable results.16 They observed the prophylactic effect of ascorbic acid supplementation on plasma thyroid hormone concentration in commercial broiler chickens. The findings of that study were similar to these results which showed a reduction in plasma thyroid levels by supplementation with dietary ascorbic acid.

**CONCLUSION**

Thyroid hormones level increased in chronic cold exposed rats so as to increase thermogenesis. However, adequate supplementation with ascorbic acid prevented the harmful effects of cold stress on the body and controlled the thyroid hormone levels within normal limits.

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