The impact of mild and short-term social stress on urinary levels of T3 and iodine in students of elementary schools

Sakineh Nouri Saeidlou1, Ehsan Saboory2,3*, Leila Derafshpour2, Sima Masudi4, Zafar Gholinejad5, Yousef Rasmi5

1. Food and Beverages Safety Research Center, Urmia University of Medical Science, Urmia, Iran
2. Neurophysiology Research Center, Urmia University of Medical Sciences, Urmia, Iran
3. Zanjan Metabolic Diseases Research Center, Zanjan University of Medical Sciences, Zanjan, Iran
4. Department of Biostatistics and Epidemiology, School of Medicine, Urmia University of Medical Science, Urmia, Iran
5. Department of Biochemistry, School of Medicine, Urmia University of Medical Science, Urmia, Iran

ABSTRACT

Introduction: Stress is a determinant factor in thyroid gland functions. A decrease in iodine concentration and thyroid hormones impairs cognitive functions in students. The responsiveness of thyroid hormones to stresses depends on many factors such as the intensity and duration of a particular stressor. This study aimed to examine the effect of mild and short-term social stress on heart rate (HR), blood pressure (BP), triiodothyronine (T3) and iodine concentrations of urine in students.

Methods: This controlled before-after study was conducted on 200 students aged 9-12 years (100 girls and 100 boys). The HR and BP of the students were measured and their urine samples were collected before and after the intervention in both stressed and control students. To induce stress, the students were asked to read aloud a text from their own book in front of some audience.

Results: In a mixed sample of girls and boys, a significant effect of stress was detected on BP and HR. There was no significant association between the stress and urinary level of T3 and iodine neither in girls nor in boys (and mixed sample). In girls, HR significantly increased in the stressed group while there was no significant connection between stress and BP. In boys, there was a significant difference between stress and control groups in terms of systolic and diastolic BP, but not the HR.

Conclusion: Although the stress had a significant sex-specific impact on HR and BP, urinary levels of T3 and iodine were not affected in elementary students.

Keywords: Blood pressure, Elementary schools, Iodine, Metabolic adjustments, Social stress, T3

Introduction

Any intrinsic or extrinsic stimulus that evokes a biological response is known as stress (Yaribeygi et al., 2017). The effects of stress are known on numerous physiological processes such as endocrine, immune, central nervous system, cardiovascular responses, psychiatric disorders (McEwen, 2000). Stressful events can disrupt the normal regulation of neuroendocrine axes. Stress can be either acute or chronic. Acute stress lasts for a period of several minutes to hours, but chronic

* Corresponding author: Ehsan Saboory, saboory@zums.ac.ir
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stress lasts for days to months and even life-long. It is expected that short-term social stress can acutely increase heart rate (HR) and blood pressure (BP), which are the primary outcomes of the current study. The stress level can determine the peak levels of stress hormones, neurotransmitters, and other physiological changes (e.g., increases in HR and BP), as well as the amount of time these changes persist during and following stressor exposure (Sephton et al., 2000). Psychological stress is more difficult to measure than the other stresses such as those associated with illness or exercise. What is stressful to one individual may not prove so to another. In this regard, physical and psychological stressors can activate the hypothalamic-pituitary-thyroid axis (Kyriacou et al., 2015). Fluctuations in thyroid hormones are linked to psychiatric disease (Baumgartner, 2000; Kyriacou et al., 2015), sexual behavior (Dellovade et al., 1996) and even evolution (Crockford, 2003). There are no conclusive results on whether stress reduces or increases thyroid hormones. An increase and decrease in thyroid hormone levels have been detected in immobilization (Turakulov et al., 1994), noise (Armarion et al., 1984), lipopolysaccharide injection (Kondo et al., 1997) and escapable tail-shock (Servatius et al., 2000). Iodine is a trace element essential for thyroid hormone production that primarily received from the diet and excreted in the urine. This element plays a major role in the growth mechanisms and the development of tissues. The iodine status in children affects their mental performance. Low urinary iodine concentration in school children (7–11 y) has a significantly greater decrease in performance on the combination of mental tests (van den Briel et al., 2000). It has been reported that adequate iodine administration improves the memory capacity and reduces the level of anxiety and stress in school children (Mogommedibirova et al., 2017). Accurate measurement of dietary iodine intake from foods and water at a population level is a difficult task. In this regard, WHO/ICCIDD/UNICEF recommends measurement of urinary iodine concentration (UIC) in evaluating population iodine nutrition (WHO, 2001). Indeed, UIC is a biomarker of goiter and thyroid functions (Zimmermann and Andersson, 2012). Although measuring UIC does not directly assess thyroid function, a deficient or an excessive median UIC in a population predicts a higher risk for the development of thyroid disorders. It is known that different stress models have different effects on thyroid function and the level of thyroid hormones. Moreover, students in the age of elementary school (7-12 y) have a rapidly developing brain. Stress affects the developing brain of these children more severely than that of adults (Saboory et al., 2019). Therefore, evaluating common stressful events at this age can be useful for expanding our understanding to better involve in teaching and growing up these students. Generally, presentations and/or giving a lecture in a meeting can be stressful even for expert adults and even might be more stressful in elementary students. This is sometimes referred to as performance anxiety (Chorpita and Barlow, 1998). In the present study, we used this method to induce stress in students. However, previous literature does not clearly relate certain effects to each specific stress model and the role that stressors may play in modulating levels of other hormones, especially during development while the brain and body continue to mature. Therefore, this work was designed to expand our current understanding in this context. This study is an attempt to assess the impact of mild and short-term social stress on urinary levels of triiodothyronine (T3) and iodine in elementary school. We hypothesized that the stress procedure alters urinary levels of T3 and iodine.

Methods and Materials

Ethical approve

The present research was approved by the Ethics Committee of the Urmia University of Medical Sciences (ID: IR.XMSU.REC.1395.435) and registered at the Iran Registry of Clinical Trials under IRCT20171015036779N2.

Participants

This controlled before-after study was conducted among elementary school students in Urmia, the capital of West Azarbyjan, Iran. A written informed consent was obtained from the parents of the students. In total, 200 students with age range of 9-12 years (100 girls and 100 boys) were selected using a simple random sampling method with replacement from the list of students in grades 4 to 6 in two elementary schools. It is of note that all the schools are single-sex in Iran. The students who had any recognized illness and drug take in the past two months, as well as family problems including parental divorce, death of parents and relatives during the past six months were not included in the study.
Randomization

To assign 100 students to the intervention groups in each school, a block randomization method with a block size of four was used to divide the students into stress and control groups. This randomization method was performed to yield a balanced number of students in two groups. The blocks and their random order were defined before the random selection of students. At each elementary school, the list of all students of fourth, fifth, and sixth grade was obtained from school officials. An identification number from 1 to N was assigned to each student. Then, using a table of random numbers, 100 students were selected from each school. We considered a 20% chance of not agreeing to participate in the study either by the students or their parents. The selection order of the students was recorded. After obtaining the informed consent from the parents of the students, we matched the selection order of the students with the predefined order of the blocked randomization to identify the group that the students were assigned to. Student characteristics including their height, weight, schooling score, age, education of their parents and their mothers’ disease history were obtained through interviews with students and their mothers, simultaneously. Mothers completed a questionnaire on their stress status based on Cohen’s perceived stress score. The HR and BP of the students were measured before and after the intervention in both stressed and control groups. All students in the stressed group were requested to provide a urine sample before the intervention and urine sample 2h after the intervention. The students in the control group provided two urine samples with a similar time course as the stress group without any intervention. All urine samples were taken between 9:00am and 12 midday.

Stress procedures

Before calling the students to the stress room, their HR and BP were measured in sitting position at the classroom in the presence of other students. Then, after 15-30min, the students were guided to the stress room two at a time. The students were asked to read aloud a text from their own book for 3min. The text and poetry were selected from a portion of the book that was unfamiliar for children (confirmed by their teacher). This procedure was performed in the school principal’s room with the presence of parents, teachers, school administrators and at least two members of the current research team. Students were called, two at a time, to a table at the front of the room. They were asked to stay quiet for the first 2min and then read aloud the selected text for 3min in standing position. Next, they again were asked to stay quiet for another 2min in sitting position. Afterward, the HR and BP of students were measured in the sitting position and their systolic, diastolic and HR were recorded (Gholami and Saboory, 2013). For measuring HR, we put the tips of our little and ring finger on the medial side of the student’s wrist and count the number of pulses per minute. Moreover, to measure blood pressure, a sphygmomanometer (Health-Ace Model: 501-V) and a stethoscope (ALP K2, Capes medical, New Zealand) were used.

Spot urine samples were collected and delivered to the laboratory for measuring UIC. The UIC was determined by the modified acid-digestion method (Dunn et al., 1993). In this study, the colorimetric ceric-arsenic assay, which yields the colorless cerium and arsenic, was used for measuring urinary iodine. This assay is a two-step reaction where the rate of the reaction is measured during the reduction of the yellow-colored ceric ions by arsenic in the presence of iodide to form the colorless cerous ions and elemental iodine.

The reduction in the yellow cerium was measured spectrophotometrically at 405nm. Some of the students were unable to provide a urine sample before the intervention or after it. Also, in some cases, the volume of the samples was not sufficient. The analysis was performed using the data of students who had two successful measures of urine iodine and T3. Totally, 138 students remained in the analysis, of which 41 girls and 32 boys were assigned to the control group and 41 girls and 24 boys to the stressed group.

Statistical analysis

Quantitative variables were reported as means and standard deviations and qualitative variables were reported in percentage. The Kolmogorov-Smirnov test was used to determine the normality of distribution. Chi-square and independent t-test were used to compare the baseline characteristics of the students in control and stressed groups. We used the one-way analysis of covariance (ANCOVA) to compare the effect of mild social stress on the outcome variables whilst controlling their baseline measurements. We used a natural log transform-
mation of the variable with non-normal distribution. Leven’s test was used to check the assumption of the equality of variances. The results showed that this assumption was met in our data. We reported the effect size of the intervention for each variable based on partial eta squares estimated in the results of analysis of covariance. An effect size equal to 0.2, 0.5 and 0.8 is considered as small, medium and large effect size, respectively. Data analysis was performed using SPSS version 20 (SPSS Inc., Chicago, IL). Differences with $P < 0.05$ were considered statistically significant.

**Results**

The baseline characteristics of the stressed and control groups are shown in Table 1. No statistically significant differences were identified between the two groups. Since, the students of both groups were in similar age and gender groups, these variables were not reported in Table 1. Several students either were unable to provide a urine sample or the volume of the samples was not sufficient. So, they were removed from the data analysis. Finally, 138 students remained in the analysis, of which 41 (63.1%) girls and 32 (36.9%) boys were assigned to the control group and 41 (56.2%) girls and 24 (43.8%) boys to the stressed group.

The results of one-way ANCOVA showed no significant difference between control and stressed groups in terms of urinary T3 and iodine concentration. But, a significant effect of stress was detected on PR, systolic and diastolic BP after controlling for their baseline measures, although the baseline values were associated with their after-stress levels ($P < 0.05$, Table 2).

In girls, there was no significant difference between stressed and control groups in terms of urinary T3 and iodine concentration, systolic and diastolic BP. The baseline measures (before stress) of these variables (except T3) were associated with after stress values of them. In comparison, stress had a significant effect on pulse rate after controlling for its baseline measures, although baseline values were associated with post-stress measures ($P < 0.05$, Table 3).

In boys, there was a significant difference between stressed and control groups in terms of systolic and diastolic BP ($P = 0.002$ and $P = 0.022$, respectively).

**TABLE 1:** Comparison of the baseline characteristics of participants between two groups.

<table>
<thead>
<tr>
<th>Variables*</th>
<th>Control</th>
<th>Stressed</th>
<th>$P$-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means ± SD</td>
<td>Means ± SD</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>146.72±10.10</td>
<td>146.61±8.71</td>
<td>0.945</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>44.72±14.4</td>
<td>44.45±13.1</td>
<td>0.911</td>
</tr>
<tr>
<td>Schooling score</td>
<td>17.61±2.3</td>
<td>17.14±3.39</td>
<td>0.363</td>
</tr>
<tr>
<td>Mother age</td>
<td>37.47±5.64</td>
<td>36.66±5.97</td>
<td>0.444</td>
</tr>
<tr>
<td>Mother stress score</td>
<td>22.96±7.09</td>
<td>22.20±7.99</td>
<td>0.577</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>41(63.1)</td>
<td>41(56.2)</td>
<td>0.409</td>
</tr>
<tr>
<td>Boys</td>
<td>32(36.9)</td>
<td>24(43.8)</td>
<td></td>
</tr>
<tr>
<td>Child’s disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>69(87.5)</td>
<td>56(94.5)</td>
<td>0.147</td>
</tr>
<tr>
<td>Yes</td>
<td>4(12.5)</td>
<td>8(5.5)</td>
<td></td>
</tr>
<tr>
<td>Drug use by children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>71(97.3)</td>
<td>60(95.2)</td>
<td>0.532</td>
</tr>
<tr>
<td>Yes</td>
<td>2(4.8)</td>
<td>3(2.7)</td>
<td></td>
</tr>
<tr>
<td>Mother’s disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>54(75.0)</td>
<td>54(87.1)</td>
<td>0.077</td>
</tr>
<tr>
<td>Yes</td>
<td>18(25.0)</td>
<td>8(12.9)</td>
<td></td>
</tr>
<tr>
<td>Drug use by the mother</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>57(79.2)</td>
<td>51(82.3)</td>
<td>0.652</td>
</tr>
<tr>
<td>Yes</td>
<td>15(20.8)</td>
<td>11(17.7)</td>
<td></td>
</tr>
<tr>
<td>Mother’s education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma or less</td>
<td>50(64.9)</td>
<td>41(67.2)</td>
<td>0.782</td>
</tr>
<tr>
<td>Collegiate</td>
<td>22(35.1)</td>
<td>20(32.8)</td>
<td></td>
</tr>
</tbody>
</table>

*Values are means ± SD, N (%)  
**$P$-values were obtained by independent sample t-test and chi-square test methods
er, stress did not show any significant effect on urinary T3 and iodine levels whilst controlling for their pre-intervention level. The baseline values of all variables were associated with the post-stress level of them (P<0.05, Table 4).

**Discussion**

In the current study, the effect of mild and short-term social stress was examined on HR, BP, urinary T3 and iodine levels in a sample of elementary school students. The social stress procedure induced only changes in cardiovascular parameters that are adaptive physiological responses. New to our study and contrary to our original hypotheses, endocrinological parameters were not affected in stressed students. In other words, stress has significant effects on BP and HR and no significant effect in terms of urinary T3 and iodine concentration.

Social stress, defined as the set of physiological stress responses specifically caused by the presence and actions of certain conspecifics, has repeatedly been shown to predict BP and cardiovascular disease (Smith et al., 2012). The association between experience of social-evaluative threat during the day and an increase in BP is proven (Kamarck et al., 2005). A pattern of autonomic activity during acute stress can be a mediator of changes of BP and HR in which both beta-adrenergic sympathetic activation and vagal withdrawal are involved to a roughly equal extent (Brindle et al., 2014). In the current study, the effect of mild and short-term social stress on BP (in boys) and HR (in girls) was statis-
TABLE 4: The results of the analysis of covariance for boy students.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Before intervention</th>
<th>After the intervention</th>
<th>Difference scores</th>
<th>Observed effect size*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>Control</td>
<td>98.34(12.56)</td>
<td>90.44(15.24)</td>
<td>-7.90</td>
<td>0.166</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>100.67(11.82)</td>
<td>100.88(132.85)</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>Control</td>
<td>62.47(9.03)</td>
<td>56.59(10.09)</td>
<td>-5.88</td>
<td>0.094</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>61.25(7.82)</td>
<td>61.58(7.82)</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse rate</td>
<td>Control</td>
<td>83.56(9.46)</td>
<td>85.19(13.05)</td>
<td>1.63</td>
<td>0.013</td>
<td>0.415</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>94.17(12.38)</td>
<td>94.29(12.88)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary iodine (µg/dl)</td>
<td>Control</td>
<td>12.09(7.50)</td>
<td>12.45(8.45)</td>
<td>0.75</td>
<td>0.057</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>12.12(7.50)</td>
<td>12.31(12.12)</td>
<td>4.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary T3 (ng/dl)</td>
<td>Control</td>
<td>4.03(1.70)</td>
<td>4.49(2.28)</td>
<td>0.46</td>
<td>0.009</td>
<td>0.497</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>4.28(2.02)</td>
<td>4.61(1.69)</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*It is reported as the partial eta square in the analysis of covariance.

Physically significant. It has been reported that in physiological responses to acute behavioral stressors, boys exhibit a significant increase in BP and sympathetic responses to a challenge compared to girls (Brindle et al., 2014). On the other hand, girls have larger heart rate responses to the videotaped speech task as an acute stressor compared to boys (Kudielka et al., 2004). Meanwhile, a study reported sex differences in both systolic and diastolic BP responses during stress among middle-aged subjects, but not among children (Matthews and Stoney, 1988). However, sex differences in BP and HR are highly dependent upon the nature of the task and stressor (Matthews and Stoney, 1988). The boys who experience annoyance or anger in school often hide these feelings; this may be the cause of elevations of BP in stress reactivity. Overall, further research is needed to understand the mechanisms linking sex to BP and HR in mild and short-term social stress. The results of this study are consistent with the findings of others confirming the six specific effects of stress in many subjects including rats and humans (Gholami and Saboory, 2013; Gholipoor et al., 2017; Saboory et al., 2019).

A decrease in the activity of the thyroid gland can be yielded through stressing agents (Bogoroch and Timiras, 1951). Namely, when cortisol levels change through a stressful situation, a negative relationship is found between cortisol and thyroid stimulating hormone (Samuels and McDaniel, 1997). However, we did not find any change of urinary T3 and iodine concentration in response to the stress. In the current study, the values of urinary iodine levels were in normal range according to WHO standards (10-19.9 µg/dl); there was no reliable reference for urinary T3 level (WHO, 2001), therefore, we could not compare it with other studies.

Nevertheless, there is a wide range of stressors to induce a decrease in T3 levels in restraint (Bianco et al., 1987), fasting (Boelen et al., 2008) and heat stress (Etchells et al., 2008). Likely, the change of T3 levels may be seen after long-term exposure to a stressor (Hangalapura et al., 2004). A slight increase or no change in peripheral thyroid hormone levels may be caused by mild stressors (Turakulov et al., 1994); which may explain our results. A mild decrease in urinary iodine content is likely following exposure to severe stress. However, no significant changes were observed in the current study after stress, probably due to the mild intensity and short duration of applied stress in our study. Acute stress may be considered as a factor activating iodide content in the thyroid gland. Nevertheless, the univocal solution to this problem requires further investigations because of the multilevel effects of glucocorticoids on thyroid homeostasis (Nadolnik, 2012). We examined the effect of acute social stress on iodine and T3 levels after 120min of stress exposure whereas the adjustment of thyroid hormone levels to a new condition may require more time. One limitation of our study is a single time course of urine sampling (120min after stress exposure). Although there are reports that 120min interval between stressor exposure and urine sample can be sufficient to detect the changes in urinary iodine levels, trying different time
courses (which was not done in this study) can be helpful. Our study had another critical limitation; we had to exclude those students from data analysis that could not complete the second urine sampling and/or those with a too low urine volume. This exclusion decreased the sample size and likely affected the obtained results.

The effects of severe iodine deficiency on neurological development have been well documented. It has been reported that chronic moderate-to-severe iodine deficiency, particularly in children, reduces IQ by about 12-13.5 points (Zimmermann, 2009). A 2004 Cochrane review concluded that iodine supplementation in children living in areas of iodine deficiency appears positively affect physical and mental development and decrease mortality with only minor and transient adverse effects (Angermayr and Clar, 2004). The effects of mild iodine deficiency during childhood are more difficult to quantify. Some studies suggest that mild iodine deficiency is associated with subtle neurodevelopmental deficits and that iodine supplementation might improve cognitive function in mildly iodine-deficient children (Melse-Boonstra and Jaiswal, 2010). In a study, 184 children aged 10-13 years in New Zealand with a median UIC of 63µg/l received iodine supplements (150µg/day) or placebo for 28 weeks (Gordon et al., 2009). Iodine supplementation improved iodine status and significantly improved measures of perceptual reasoning and overall cognitive score compared with children taking a placebo. These findings suggest that correcting mild iodine deficiency in children could improve certain components of cognition. In the current study, students’ learning score was obtained from their schools but there was no correlation between the scores and iodine and T3 levels (data not shown). The current study is unique from this point of view that we studied elementary school students with both sexes (girls and boys) simultaneously in the same region of a city (homogeny sample) and with exactly the same method, separately. To the best of authors’ knowledge, in most parts of the world, elementary schools are mixed-sex education-based. But, in Iran, all the schools, including elementary schools, are single-sex. Thus, this condition as a strength of this study lets us examine the girls and boys in separate environments.

Conclusion

It can be concluded that exposure to mild and short-term stress such as a presentation in a small meeting has sufficient power to activate the stress response in elementary students, as shown by increased HR and BP. However, the intensity of such stressors is not sufficient to produce detectable changes in the urinary content of iodine and T3. We suggest conducting similar studies with greater sample size and different time courses (e.g., 60-240min) between stress exposure and urine sampling.

Conflict of interest

The authors declare no conflicts of interest.

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